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| <b>(21) International Application Number:</b> PCT/US96/19445<br><b>(22) International Filing Date:</b> 10 December 1996 (10.12.96)<br><br><b>(30) Priority Data:</b><br>60/008,582                      13 December 1995 (13.12.95)      US<br>60/018,962                      6 June 1996 (06.06.96)                      US<br><br><b>(71) Applicant (for all designated States except US):</b> MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).<br><br><b>(72) Inventors; and</b><br><b>(75) Inventors/Applicants (for US only):</b> ARENA, Joseph, P. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). CULLY, Doris, F. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). FEIGHNER, Scott, D. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). HOWARD, Andrew, D. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). LIBERATOR, Paul, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). SCHAEFFER, James, M. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). VAN DER PLOEG, Leonardus [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). |           | <b>(74) Common Representative:</b> MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).<br><br><b>(81) Designated States:</b> CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).<br><br><b>Published</b><br><i>With international search report.</i><br><i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> |
| <b>(54) Title:</b> GROWTH HORMONE SECRETAGOGUE RECEPTOR FAMILY<br><br><b>(57) Abstract</b><br><br>Human, swine and rat growth hormone secretagogue receptors have been isolated, cloned and sequenced. Growth hormone secretagogue receptors are new members of the G-protein family of receptors. The growth hormone secretagogue receptors may be used to screen and identify compounds which bind to the growth hormone secretagogue receptor. Such compounds may be used in the treatment of conditions which occur when there is a shortage of growth hormone, such as observed in growth hormone deficient children, elderly patients with musculoskeletal impairment and recovering from hip fracture and osteoporosis.   |           |  |

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TITLE OF THE INVENTION

GROWTH HORMONE SECRETAGOGUE RECEPTOR FAMILY

FIELD OF THE INVENTION

5           This invention relates to a new family of receptors, which includes the growth hormone secretagogue receptors (GHSRs) and growth hormone secretagogue-related receptors (GHSRRs), nucleic acids encoding these receptors; and to the use of a GHSR to identify growth hormone secretagogues and compounds that modulate GHSR  
10           function.

BACKGROUND OF THE INVENTION

          Growth hormone (GH) is an anabolic hormone capable of promoting linear growth, weight gain and whole body nitrogen  
15           retention. Classically, GH is thought to be released primarily from the somatotroph cells of the anterior pituitary under the coordinate regulation of two hypothalamic hormones, growth hormone releasing factor (GHRF or GRF) and somatostatin. Both GHRF stimulation and somatostatin inhibition of the release of GH occurs by the specific  
20           engagement of receptors on the cell membrane of the somatotroph.

          Recent evidence has been mounting which suggests that GH release is also stimulated by a group of short peptides, the growth hormone releasing peptides (GHRP; GHRP-6, GHRP-2 [hexarelin]) which are described, for example, in U.S. Patent No. 4,411,890, PCT  
25           Patent Pub. No. WO 89/07110, PCT Patent Pub. No. WO 89/07111, PCT Patent Pub. No. WO 93/04081, and *J. Endocrinol Invest.*, 15 (Suppl 4), 45 (1992). These peptides function by selectively binding to distinct somatotroph cell membrane receptor, the growth hormone secretagogue receptor(s) (GHSRs). A medicinal chemical approach has  
30           resulted in the design of several classes of orally-active, low molecular weight, non-peptidyl compounds which bind specifically to this receptor and result in the pulsatile release of GH. Such compounds possessing growth hormone secretagogue activity are disclosed, for example, in the following: U.S. Patent No. 3,239,345; U.S. Patent No. 4,036,979; U.S.

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Patent No. 4,411,890; U.S. Patent No. 5,206,235; U.S. Patent No. 5,283,241; U.S. Patent No. 5,284,841; U.S. Patent No. 5,310,737; U.S. Patent No. 5,317,017; U.S. Patent No. 5,374,721; U.S. Patent No. 5,430,144; U.S. Patent No. 5,434,261; U.S. Patent No. 5,438,136; U.S. Patent No. 5,494,919; U.S. Patent No. 5,494,920; U.S. Patent No. 5,492,916; EPO Patent Pub. No. 0,144,230; EPO Patent Pub. No. 0,513,974; PCT Patent Pub. No. WO 94/07486; PCT Patent Pub. No. WO 94/08583; PCT Patent Pub. No. WO 94/11012; PCT Patent Pub. No. WO 94/13696; PCT Patent Pub. No. WO 94/19367; PCT Patent Pub. No. WO 95/03289; PCT Patent Pub. No. WO 95/03290; PCT Patent Pub. No. WO 95/09633; PCT Patent Pub. No. WO 95/11029; PCT Patent Pub. No. WO 95/12598; PCT Patent Pub. No. WO 95/13069; PCT Patent Pub. No. WO 95/14666; PCT Patent Pub. No. WO 95/16675; PCT Patent Pub. No. WO 95/16692; PCT Patent Pub. No. WO 95/17422; PCT Patent Pub. No. WO 95/17423; PCT Patent Pub. No. WO 95/34311; PCT Patent Pub. No. WO 96/02530; *Science*, 260, 1640-1643 (June 11, 1993); *Ann. Rep. Med. Chem.*, 28, 177-186 (1993); *Bioorg. Med. Chem. Ltrs.*, 4(22), 2709-2714 (1994); and *Proc. Natl. Acad. Sci. USA* 92, 7001-7005 (July 1995).

The use of orally-active agents which stimulate the pulsatile release of GH would be a significant advance in the treatment of growth hormone deficiency in children and adults as well as provide substantial benefit under circumstances where the anabolic effects of GH might be exploited clinically (e.g. post-hip fracture rehabilitation, the frail elderly and in post-operative recovery patients).

It would also be desirable to know the molecular structure of growth hormone secretagogue receptors in order to analyze this new receptor family and understand its normal physiological role in concert with the actions of GHRF and somatostatin. This could lead to a better understanding of the *in vivo* processes which occur upon ligand-receptor binding. Further, it would be desirable to use cloned-growth hormone secretagogue receptors as essential components of an assay system which can identify new growth hormone secretagogues.

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### DETAILED DESCRIPTION OF THE INVENTION

This invention relates to a novel family of receptors which includes growth hormone secretagogue receptors (GHSRs) and growth hormone secretagogue-related receptors (GHSRRs).

5

A first aspect of this invention are the growth hormone secretagogue receptors, which are free from receptor associated proteins. GHSRs may be from any species, and in further embodiments may be isolated or purified. One embodiment of this invention is

1 0 human growth hormone secretagogue receptor (hGHSR), free from receptor-associated proteins. A further aspect of this invention is hGHSR which is isolated or purified.

Another aspect of this invention is swine growth hormone secretagogue receptor (sGHSR), free from receptor-associated proteins.

1 5 A further aspect of this invention is sGHSR which is isolated or purified.

Another aspect of this invention is rat growth hormone secretagogue receptor (rGHSR), free from receptor-associated proteins. A further aspect of this invention is rGHSR which is isolated or

2 0 purified.

Another aspect of this invention are human, swine and rat GHSRs which are encoded by substantially the same nucleic acid sequences, but which have undergone changes in splicing or other RNA processing-derived modifications or mutagenesis induced changes, so

2 5 that the expressed protein has a homologous, but different amino acid sequence from the native forms. These variant forms may have different and/or additional functions in human and animal physiology or *in vitro* in cell based assays.

Another aspect of this invention are the growth hormone

3 0 secretagogue-related receptors, free from associated receptor proteins. A further embodiment are GHSRRs which are isolated or purified. These may be from any species, including human, mouse, rat and swine.

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Growth hormone secretagogue receptors are proteins containing various functional domains, including one or more domains which anchor the receptor in the cell membrane, and at least one ligand binding domain. As with many receptor proteins, it is possible to  
5 modify many of the amino acids, particularly those which are not found in the ligand binding domain, and still retain at least a percentage of the biological activity of the original receptor. In accordance with this invention, it has been shown that the N-terminal portions of the GHSR are not essential for its activation by the Growth Hormone  
10 Secretagogues (GHSs). Thus this invention specifically includes modified functionally equivalent GHSRs which have deleted, truncated, or mutated N-terminal portions. This invention also specifically includes modified functionally equivalent GHSRs which contain modified and/or deletions in other domains, which are not accompanied  
15 by a loss of functional activity.

Additionally, it is possible to modify other functional domains such as those that interact with second messenger effector systems, by altering binding specificity and/or selectivity. Such functionally equivalent mutant receptors are also within the scope of this  
20 invention.

A further aspect of this invention are nucleic acids which encode a growth hormone secretagogue receptor or a functional equivalent from swine, human, rat or other species. These nucleic acids may be free from associated nucleic acids, or they may be isolated or  
25 purified. For most cloning purposes, cDNA is a preferred nucleic acid, but this invention specifically includes other forms of DNA as well as RNAs which encode a GHSR or a functional equivalent.

Yet another aspect of this invention relates to vectors which comprise nucleic acids encoding a GHSR or a functional equivalent.  
30 These vectors may be comprised of DNA or RNA; for most cloning purposes DNA vectors are preferred. Typical vectors include plasmids, modified viruses, bacteriophage and cosmids, yeast artificial chromosomes and other forms of episomal or integrated DNA that can encode a GHSR. It is well within the skill of the ordinary artisan to

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determine an appropriate vector for a particular gene transfer or other use.

A further aspect of this invention are host cells which are transformed with a gene which encodes a growth hormone secretagogue receptor or a functional equivalent. The host cell may or may not naturally express a GHSR on the cell membrane. Preferably, once transformed, the host cells are able to express the growth hormone secretagogue receptor or a functional equivalent on the cell membrane. Depending on the host cell, it may be desirable to adapt the DNA so that particular codons are used in order to optimize expression. Such adaptations are known in the art, and these nucleic acids are also included within the scope of this invention. Generally, mammalian cell lines, such as COS, HEK-293, CHO, HeLa, NS/0, CV-1, GC, GH3 or VERO cells are preferred host cells, but other cells and cell lines such as *Xenopus* oocytes or insect cells, may also be used.

Growth hormone secretagogue related receptors are related to GHRS, but are encoded by a distinct gene. The GHRR genes may be identified by hybridization (using relaxed or moderate stringency post-hybridizational washing conditions) of cDNA of GHR DNA to genomic DNA. These sequences have a high degree of similarity to GHR.

Another aspect of this invention is a process for identifying nucleic acids encoding growth hormone secretagogue related receptors comprising hybridizing a first nucleic acid encoding a growth hormone secretagogue receptor with a second nucleic acid suspected of comprising nucleic acids encoding a growth hormone secretagogue, wherein the hybridizing takes place under relaxed or moderate post hybridizational washing conditions; and identify areas of the second nucleic acid where hybridization occurred.

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### BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 is the DNA of Swine GHSR (type I) contained in Clone 7-3.

5      FIGURE 2 is the amino acid sequence of swine GHSR encoded by the DNA of Figure 1.

FIGURE 3 is the entire open reading frame of the type I clone of Figure 1.

FIGURE 4 is the DNA of Swine GHSR (type II) contained in Clone 1375.

10      FIGURE 5 is the amino acid sequence of swine GHSR (type II) encoded by the DNA of Figure 4.

FIGURE 6 is the DNA for human GHSR (Type I) contained in Clone 1146.

15      FIGURE 7 is the amino acid sequence of human GHSR (type I) encoded by the DNA of Figure 6.

FIGURE 8 is the entire open reading frame of Type I GHSR, encoded by the DNA sequence of Figure 6.

FIGURE 9 is the DNA for human GHSR (type II) contained in Clone 1141.

20      FIGURE 10 is the amino acid sequence of human GHSR (Type II) encoded by Clone 1141.

FIGURE 11 is the DNA for human GHSR (Type I) contained in Clone 1143.

25      FIGURE 12 is the amino acid sequence of human GHSR (Type I) encoded by Clone 1143.

FIGURE 13 compares the ORF of swine Type I (lacking the MET initiator of the full length GHSR and lacking 12 additional amino acids) to the homologous domain of swine Type II receptors.

30      FIGURE 14 compares the homologous domain of human Type I and Type II receptors (the amino terminal sequence lacks the MET initiator and four additional amino acids).

FIGURE 15 compares the ORFs of swine Type I and human Type I receptors (the amino terminal sequence lacks the MET initiator and 12 additional amino acids).

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FIGURE 16 compares full length swine Type II and human Type II receptors.

FIGURE 17 is a schematic diagram depicting the physical map of swine and human growth hormone secretagogue receptor cDNA clones.

FIGURE 18 is a graph demonstrating the pharmacology of the expressed swine and human growth hormone secretagogue receptors in *Xenopus* oocytes using the aequorin bioluminescence assay.

FIGURE 19 is a table demonstrating the pharmacology of the expressed swine and human growth hormone secretagogue receptors in *Xenopus* oocytes using the aequorin bioluminescence assay and various secretagogues.

FIGURE 20 is a graph representing the pharmacology of the pure expressed swine growth hormone secretagogue receptor in COS-7 cells using the <sup>35</sup>S-labeled Compound A binding assay.

FIGURE 21 is a table representing the competition analysis with the pure expressed swine growth hormone secretagogue receptor in COS-7 cells using the <sup>35</sup>S-labeled Compound A binding assay and various secretagogues and other G-protein coupled-receptors (GPC-Receptors) ligands in a competition assay.

FIGURE 22 is the amino acid sequence of the full length human GHSR (type I) encoded by clone 11304.

FIGURE 23 is the rat GHSR DNA sequence from the Met Initiation codon to the Stop codon. This sequence includes an intron.

FIGURE 24 is the open reading frame only of the rat GHSR of Figure 23.

FIGURE 25 is the deduced amino acid sequence of the ORF of Figure 24.

FIGURE 26 shows the expression of functional rat GHSR in transfected HEK-293 cells.

As used throughout the specification and claims, the following definitions shall apply:

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Growth Hormone Secretagogue - any compound or agent that directly or indirectly stimulates or increases the release of growth hormone in an animal.

5       Ligands-- any molecule which binds to GHSR of this invention. These ligands can have either agonist, partial agonist, partial antagonist or antagonist activity.

Free from receptor-associated proteins-- the receptor protein is not in a mixture or solution with other membrane receptor proteins.

10       Free from associated nucleic acids-- the nucleic acid is not covalently linked to DNA which it is naturally covalently linked in the organism's chromosome.

Isolated receptor--the protein is not in a mixture or solution with any other proteins.

15       Isolated nucleic acid-- the nucleic acid is not in a mixture or solution with any other nucleic acid.

Functional equivalent--a receptor which does not have the exact same amino acid sequence of a naturally occurring growth hormone secretagogue receptor, due to alternative splicing, deletions, mutations, or additions, but retains at least 1%, preferably 10%, and more preferably 25% of the biological activity of the naturally occurring receptor. Such derivatives will have a significant homology with a natural GHSR and can be detected by reduced stringency hybridization with a DNA sequence obtained from a GHSR. The nucleic acid encoding a functional equivalent has at least about 50% homology at the nucleotide level to a naturally occurring receptor nucleic acid.

Purified receptor-- the receptor is at least about 95% pure.

Purified nucleic acid-- the nucleic acid is at least about 95% pure.

30       Compound A -- (N-[1(R)-[(1,2-dihydro-1-methane-sulfonylspiro[3H-indole-3,4'-piperidin]-1'-yl)carbonyl]-2-(phenyl-methyloxy)ethyl]-2-amino-2-methyl propanamide, described in Patchett, 1995 *Proc. Natl. Acad. Sci.* 92:7001-7005.

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Compound B -- 3-amino-3-methyl-N-(2,3,4,5-tetra-hydro-2-oxo-1-([2'-1H-tetrazol-5-yl)(1,1'-biphenyl)-4-yl]methyl)-1H-benzazepin-3(R)yl-butanamide, described in Patchett, 1995 *Proc. Natl. Acad. Sci.* 92:7001-7005.

- 5           Compound C -- 3-amino-3-methyl-N-(2,3,4,5-tetrahydro-2-oxo-1-([2'-1H-tetrazol-5-yl)(1,1'-biphenyl)-4-yl]methyl)-1H-benzazepin-3(S)yl-butanamide, described in U.S. Patent 5,206,235.

Standard or high stringency post hybridizational washing conditions -- 6 X SSC at 55°C

- 10           Moderate post hybridizational washing conditions -- 6 X SSC at 45°C

Relaxed post hybridizational washing conditions -- 6 X SSC at 30°C

- 15           The proteins of this invention were found to have structural features which are typical of the 7-transmembrane domain (TM) containing G-protein linked receptor superfamily (GPC-R's or 7-TM receptors). Thus growth hormone secretagogue family of receptors make up new members of the GPC-R family of receptors. The intact  
20           GHSRs of this invention were found to have the general features of GPC-R's, including seven transmembrane regions, three intra- and extracellular loops, and the GPC-R protein signature sequence. The TM domains and GPC-R protein signature sequence are noted in the protein sequences of the Type I GHS receptor in Figures 3 and 8. Not all  
25           regions are required for functioning, and therefore this invention also comprises functional receptors which lack one or more non-essential domains.

- 30           The GHSRs of this invention share some sequence homology with previously cloned GPC-receptors including the rat and human neurotensin receptor (approximately 32% identity) and the rat and human TRH receptor (approximately 30% identity).

The GHSRs of this invention were isolated and characterized using expression cloning techniques in *Xenopus* oocytes.

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The cloning was made difficult by three factors. First, prior to this invention, there was very little information available about the biochemical characteristics and intracellular signaling/effector pathways of the proteins. Thus, cloning approaches which depended on the use of protein sequence information for the design of degenerate oligonucleotides to screen cDNA libraries or utilize PCR could not be effectively utilized. In accordance with this invention, therefore, receptor bioactivity needed to be determined.

Secondly, the growth hormone secretagogue receptor does not occur in abundance-- it is present on the cell membrane in about 10 fold less concentration than most other membrane receptors. In order to successfully clone the receptors in accordance with this invention, exhaustive precautions had to be taken to ensure that the GHSR was represented in a cDNA library to be screened. This required isolation of intact, undegraded and pure poly (A)<sup>+</sup> mRNA, and optimization of cDNA synthesis to maximize the production of full-length molecules. In addition, a library of larger size than normal needed to be screened (approximately 0.5 to 1 x 10<sup>7</sup> clones) to increase the probability that a functional cDNA clone may be obtained.

Thirdly, no permanent cell line which expresses this receptor is known. Therefore, primary pituitary tissue had to be used as a source for mRNA or protein. This posed an additional obstacle because most primary tissues express lower amounts of a given receptor than an immortalized cell line that may be maintained in tissue culture or some tumor materials. Further, the surgical removal of a pig pituitary and extraction of biologically-active intact mRNA for the construction of a cDNA expression library is considerably more difficult than the extraction of mRNA from a tissue culture cell line. Along with the need to obtain fresh tissue continuously, there are problems associated with its intrinsic inter-animal and inter-preparation variability. The development of cell lines expressing a receptor of this invention is therefore a significant aspect of this invention.

Yet another aspect of this invention is the development of an extremely sensitive, robust, reliable and high-throughput screening

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assay which could be used to identify portions of a cDNA library containing the receptor. This assay is described and claimed in co-pending patent applications Serial No. 60/008,584, filed December 13, 1995, and Attorney Docket No. 19590PV2 filed herewith.

5 Briefly, the ability to identify cDNAs which encode growth hormone secretagogue receptors depended upon two discoveries made in accordance with this invention: 1) that growth hormone secretagogue receptor-ligand binding events are transduced through G proteins; and 2) that a particular G protein subunit,  $G_{\alpha 11}$ , must be present in the cells  
10 in order to detect receptor activity. Only when these two discoveries were made could an assay be devised to detect the presence of GHSR-encoding DNA sequences.

When the GHSR is bound by ligand (a growth hormone secretagogue), the G-proteins present in the cell activate  
15 phosphatidylinositol-specific phospholipase C (PI-PLC), an enzyme which releases intracellular signaling molecules (diacylglycerol and inositol triphosphate), which in turn start a cascade of biochemical events that promote calcium mobilization. This can be used as the basis of an assay. A detector molecule which can respond to changes in  
20 calcium concentrations, such as aequorin, a jellyfish photoprotein, is introduced into a cell along with a complex pool of up to 10,000 individual RNAs from a cDNA expression library, at least one of which may encode a GHSR. The cell is then exposed to a known growth hormone secretagogue, such as Compound A or Compound B. If one or  
25 more RNAs encodes a GHSR, then the secretagogue ligand will bind the receptor, G-protein will be activated, the calcium level will fluctuate, and the aequorin will produce measurable bioluminescence. Once a positive result is found, the procedure can be repeated with a subdivision of the RNA pool (for example, approximately 1,000, then  
30 approximately 500, then approximately 50, and then pure clones) until a single clone is identified from which RNA can be generated which encodes a GHSR.

Using this general protocol in *Xenopus* oocytes with a swine cDNA expression library, Clone 7-3 was identified as containing

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nucleic acid encoding a swine GHSR. The insert of the cDNA clone is approximately 1.5 kb in size, and downstream from the presumed initiator methionine (MET), contains an open reading frame (ORF) encoding 302 amino acids ( $M_r = 34,516$ ). The DNA and deduced amino acid sequence are given in FIGURES 1 and 2. When hydropathy analysis (e.g. Kyte-Doolittle; Eisenberg, Schwartz, Komaron and Wall) is performed on the protein sequence of clone 7-3, only 6 predicted transmembrane domains are present downstream of the presumed MET initiator. Translation of the longest ORF encoded in clone 7-3 encodes a protein of 353 amino acids ( $M_r = 39,787$ ); however an apparent MET initiator cannot be identified for this longer reading frame (FIGURE 3). This longer reading frame is significant since 7 transmembrane segments are encoded in the 353 amino acids protein in which a MET translation initiation codon located upstream of TM1 is absent. In addition, this longer protein also shares homology with known G-protein coupled receptors in its predicted TM1 domain (FIGURE 3 and next sections). Thus, clone 7-3 while truncated at its amino terminus, is fully functional, demonstrating that clone 7-3 is but one embodiment of a functional equivalent of a native GHSR.

The resultant cDNA clone (or shorter portions of, for instance only 15 nucleotides long) may be used to probe libraries under hybridization conditions to find other receptors which are similar enough so that the nucleic acids can hybridize, and is particularly useful for screening libraries from other species. Using this procedure, additional human, swine, and rat GHSR cDNAs have been cloned and their nucleotide sequences determined. Further, hybridization of a cDNA to genomic DNA demonstrated that the Type I receptor (see below) is encoded by a single gene that is highly conserved. Human, monkey, rat, mouse, dog, cow, chicken and invertebrate DNA all yielded a single hybridizing species at high stringency post-hybridization conditions. Therefore, this invention is not limited to any particular species.

A swine pituitary library, a human pituitary library, and a rat pituitary library were hybridized with a radiolabeled cDNA derived

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from the open reading frame of the swine GHSR clone 7-3. 21 positive human GHSR cDNA clones were isolated and five swine library pools yielded a strong hybridization signal and contained clones with inserts larger than clone 7-3, as judged by their insert size on Southern blots.

5 A single rat cDNA clone was also isolated.

Nucleotide sequence analysis revealed two types of cDNAs for both the human and swine GHSR cDNAs. The first (Type I) encodes a protein represented by clone 7-3, encoding seven transmembrane domains. The full length open reading frame appears to  
10 extend 13 amino acids beyond the largest predicted open reading frame of clone 7-3 (353 amino acids). The second (type II) diverges in its nucleotide sequence from the type I cDNA at its 3'-end, just after the predicted second amino acid of the sixth transmembrane domain (TM-6).

1-5 In the type II cDNAs, TM-6 is truncated and fused to a short contiguous reading frame of only 24 amino acids, followed by a translation stop codon. Swine clone 1375 is an example of a Type II cDNA (FIGURES 4 and 5). These 24 amino acids beyond TM-6 are highly conserved when compared between human and swine cDNAs.  
20 The DNA and amino acid sequences of the human GHSR Type I and II are given in FIGURES 6-12. A full length cDNA encoding the human Type I receptor, that is, a molecule encoding 7-TM domains with an initiator MET in a favorable context preceded by an inframe termination codon is isolated, and termed clone 11304. The predicted  
25 ORF of clone 11304 for the full length Type I GHSR measures 366 amino acids ( $M_r=41,198$ ; FIGURE 22). The full length human Type II cDNA encodes a polypeptide of 289 amino acids ( $M_r=32,156$ ; FIGURES 9 and 10).

30 Sequence alignments performed at both the nucleic acid and protein levels show that Type I and II GHSR's are highly related to each other and across species (FIGURES 13-16). The human and swine GHSR sequences are 93% identical and 98% similar at the amino acid level.

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The nucleotide sequence encoding the missing amino terminal extension of swine Type I clone 7-3 is derived from the predicted full length human Type I clone and the human and swine Type II cDNAs. The reading frame of the full length clones extended 13  
5 amino acids beyond the amino terminal sequence of clone 7-3 and this sequence was conserved in 12/13 amino acid residues, when compared between human and swine. The amino terminal extension includes a translation initiator methionine in a favorable context according to Kosak's rule, with the reading frame further upstream being interrupted  
10 by a stop codon. A schematic physical map of Type I and II swine and human cDNA clones is given in FIGURE 17.

The rat clone was also further investigated. Sequence analysis revealed the presence of a non-coding intronic sequence at nt 790 corresponding to a splice-donor site (see FIGURES 23, 24, and 25).  
15 The G/GT splice-donor site occurs two amino acids after the completion of the predicted transmembrane domain 5 (leucine 263), thus dividing the rGHSR into an amino-terminal segment (containing the extracellular domain, TM-1 through TM-5, and the first two intra- and extra-cellular loops) and a carboxy-terminal segment (containing TM-6, TM-  
20 7, the third intra- and extra- cellular loops, and the intra- cellular domain). The point of insertion and flanking DNA sequence are highly conserved, and also present in both human and swine Type I and II cDNAs.

Comparison of the complete open reading frame encoding  
25 the rat GHSR protein to human and swine homologs reveals a high degree of sequence identity (rat vs. human, 95.1%; rat vs. swine 93.4%.

The human GHSR can be assigned by fluorescent *in situ* hybridization analysis [FISH; as described in *Cytogenet, Cell Genet* 69: 196 (1995)] to the cytogenetic band 3Q26.2. The mouse gene is located  
30 on 3A3.

Human and swine Type I cRNAs expressed in oocytes were functional and responded to Compound A concentrations ranging from 1 mM to as low as 0.1 nM in the aequorin bioluminescence assay. Human or swine Type II-derived cRNAs that are truncated in TM-6

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failed to give a response when injected into oocytes and these represent a receptor subtype which may bind the GHS, but cannot effectively activate the intracellular signal transduction pathway. In addition the type II receptor may interact with other proteins and thus reconstitute a functional GHSR. Proteins such as these which may have ligand-binding activity, but are not active in signal transduction are particularly useful for ligand-binding assays. In these cases, one may also over-express a mutant protein on the cell membrane and test the binding abilities of putative labeled ligands. By using a non-signaling mutant which is constitutively in a high affinity state, binding can be measured, but no adverse metabolic consequences would result. Thus non-signaling mutants are an important aspect of this invention.

The pharmacological characterization of human, Type I swine, Type I and rat receptors in the aequorin bioluminescence assay in oocytes is summarized in FIGURES 18, 19, and 26. Peptidyl and non-peptidyl bioactive GHS's were active in a similar rank order of potency as observed for the native pituitary receptor. Independent confirmatory evidence that the Type I GHSR (shown for swine clone 7-3) encodes a fully-functional GHSR is given by the finding that when clone 7-3 is expressed transiently in mammalian COS-7 cells, high affinity ( $K_D \sim 0.2$  nM), saturable ( $B_{max} \sim 80$  fmol/mg protein) and specific binding ( $> 90$  % displaced by 50 nM unlabeled Compound A) is observed for  $^{35}S$ -Compound A (FIGURES 20 and 21).

The GHSR receptors of this invention may be identified by hybridization of a GHSR cDNA to genomic DNA, under relaxed or moderate post hybridizational washing conditions. This analysis yields a discreet number of hybridizing bands. A suitable human genomic library which can be used in this procedure is PAC (as described in *Nature Genetics* 6:84 (1994)) and a suitable mouse genomic library is BAC (as described in *Proc Natl Acad Sci USA* 89: 8794 (1992)).

Due to the high degree of homology to GHSRs, the GHSRs of this invention are believed to function similarly to GHSRs and have similar biological activity. They are useful in understanding the biological and physiological pathways involved in an organisms growth.

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They may be also used to scan for growth hormone secretagogue agonists and antagonists; as in particular to test the specificity of identified ligands.

- Heterotrimeric G proteins, consisting of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits, serve to relay information from cell surface receptors to intracellular effectors, such as phospholipase C and adenylate cyclase. The G-protein  $\alpha$  subunit is an essential component of the intracellular signal transduction pathway activated by receptor-ligand interaction. In the process of ligand-induced GPCR activation, the  $G_\alpha$  subunit of a trimeric G $\alpha\beta\gamma$  exchanges its bound GDP for GTP and dissociate from the  $\beta\gamma$  heterodimer. The dissociated subunit serves as the active signal transducer, often in concert with the  $\beta\gamma$  complex, thus starting the activation of the intracellular signal transduction pathway. By definition, cell surface receptors which couple intracellularly through G protein interactions are termed GPC-R's. This interaction has mainly been characterized with respect to the type of G- $\alpha$  ( $G_\alpha$ ) subunit which is primarily involved in the signal transduction process.  $G_\alpha$  subunits are classified into sub-families based on sequence identity and the main type of effectors to which they are coupled have been characterized:  $G_s$ , activate adenylate cyclase;  $G_{i/o/t}$ , inhibit adenylate cyclase;  $G_{q/11}$ , activate PI-PLC; and  $G_{12/13}$ , effector unknown.

- Expression of several receptors in heterologous cells has been shown to be increased by the co-expression of certain  $G_\alpha$  subunits. This observation formed the basis for the rationale to the use of  $G_\alpha$  subunits of several sub-families in conjunction with a source of GHSR (swine poly[A<sup>+</sup>] mRNA) to test if a GHS-induced functional response could be measured in the *Xenopus* oocyte system. GHS-induced responses were detected and were found to be strictly dependent on  $G_{\alpha 11}$  co-expression in this system, an unprecedented finding outlining the specificity of the interaction. Thus another aspect of this invention is a method of detecting a GHS response comprising co-expressing a  $G_{\alpha 11}$  protein subunit in a cell also expressing a GHSR, exposing the cell to a GHS, and detecting the response.

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Ligands detected using assays described herein may be used in the treatment of conditions which occur when there is a shortage of growth hormone, such as observed in growth hormone deficient children, elderly patients with musculoskeletal impairment and recovering from hip fracture, and osteoporosis.

5 The GHSR and fragments are immunogenic. Thus, another aspect of this invention is antibodies and antibody fragments which can bind to GHSR or a GHSR fragment. These antibodies may be monoclonal antibodies and produced using either hybridoma technology or recombinant methods. They may be used as part of assay systems or  
10 to deduce the function of a GHSR present on a cell membrane.

A further aspect of this invention are antisense oligonucleotides nucleotides which can bind to GHSR nucleotides and modulate receptor function or expression.

15 A further aspect of this invention is a method of increasing the amount of GHSRs on a cell membrane comprising, introducing into the cell a nucleic acid encoding a GHSR, and allowing expression of the GHSR.

A GHS receptor, preferably immobilized on a solid support,  
20 may be used diagnostically for the determination of the concentration of growth hormone secretagogues, or metabolites thereof, in physiological fluids, e.g., body fluids, including serum, and tissue extracts, as for example in patients who are undergoing therapy with a growth hormone secretagogue.

25 The administration of a GHS receptor to a patient may also be employed for purposes of: amplifying the net effect of a growth hormone secretagogue by providing increased downstream signal following administration of the growth hormone secretagogue thereby diminishing the required dosage of growth hormone secretagogue; or  
30 diminishing the effect of an overdosage of a growth hormone secretagogue during therapy.

The following, non-limiting Examples are presented to better illustrate the invention.

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### EXAMPLE 1

#### Oocyte Preparation and Selection

*Xenopus laevis* oocytes were isolated and injected using standard methods previously described by Arena, *et al.*, 1991, *Mol. Pharmacol.* 40, 368-374, which is hereby incorporated by reference. Adult female *Xenopus laevis* frogs (purchased from Xenopus One, Ann Arbor, MI) were anesthetized with 0.17% tricaine methanesulfonate and the ovaries were surgically removed and placed in a 60 mm culture dish (Falcon) containing OR-2 medium without calcium (82.5 mM NaCl, 2 mM KCl, 2.5 mM sodium pyruvate, 1 mM MgCl<sub>2</sub>, 100 m/ml penicillin, 1 mg/ml streptomycin, 5 mM HEPES, pH=7.5; ND-96 from Specialty Media, NJ). Ovarian lobes were broken open, rinsed several times, and oocytes were released from their sacs by collagenase A digestion (Boehringer-Mannheim; 0.2% for 2-3 hours at 18°C) in calcium-free OR-2. When approximately 50% of the follicular layers were removed, Stage V and VI oocytes were selected and placed in ND-86 with calcium (86 mM NaCl, 2 mM KCl, 1 mM MgCl<sub>2</sub>, 1.8 mM CaCl<sub>2</sub>, 2.5 mM sodium pyruvate, 0.5 mM theophylline, 0.1 mM gentamycin, 5 mM HEPES [pH=7.5]). For each round of injection, typically 3-5 frogs were pre-tested for their ability to express a control G-protein linked receptor (human gonadotropin-releasing hormone receptor) and show a robust phospholipase C intracellular signaling pathway (incubation with 1% chicken serum which promotes calcium mobilization by activation of phospholipase C). Based on these results, 1-2 frogs were chosen for library pool injection (50 nl of cRNA at a concentration of 25 ng (complex pools) to 0.5 ng (pure clone) per oocyte usually 24 to 48 hours following oocyte isolation.

### EXAMPLE 2

#### mRNA Isolation

Total RNA from swine (50-80 kg, Yorkshire strain) pituitaries (snap-frozen in liquid nitrogen within 1-2 minutes of animal sacrifice) was prepared by a modified phenol:guanidinium thiocyanate

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procedure (Chomczynski, *et al.*, 1987 *Anal. Biochem.* 162:156-159, using the TRI-Reagent LS as per the manufacturer's instructions (Molecular Research Center, Cincinnati, OH). Typically, 5 mg of total RNA was obtained from 3.5 g wet weight of pituitary tissue. Poly (A)<sup>+</sup> RNA was isolated from total RNA by column chromatography (two passes) on oligo (dT) cellulose (Pharmacia, Piscataway, NJ). The yield of poly (A)<sup>+</sup> mRNA from total RNA was usually 0.5%. RNA from other tissues was isolated similarly.

10

### EXAMPLE 3

#### cDNA Library Construction

First-strand cDNA was synthesized from poly (A)<sup>+</sup> mRNA using M-MLV RNase (-) reverse transcriptase (Superscript, GIBCO-BRL, Gaithersburg, MD) as per the manufacturer's instructions with an oligo (dT)/Not I primer-adaptor. Following second-strand cDNA synthesis, double-stranded cDNA was subjected to the following steps: 1) ligation to EcoR I adapters, 2) Not I digestion, and 3) enrichment for large cDNAs and removal of excess adapters by gel filtration chromatography on a Sephacryl S-500 column (Pharmacia). Fractions corresponding to high molecular weight cDNA were ligated to EcoR I/Not I digested pSV-7, a eucaryotic expression vector capable of expressing cloned cDNA in mammalian cells by transfection (driven by SV-40 promoter) and in oocytes using *in vitro* transcripts (initiated from the T7 RNA polymerase promoter). pSV-7 was constructed by replacing the multiple cloning site in pSG-5 (Stratagene, La Jolla, CA; Green, S. *et al.*, 1988 *Nucleic Acids Res.* 16:369), with an expanded multiple cloning site. Ligated vector:cDNA was transformed into *E.coli* strain DH10B (GIBCO-BRL) by electroporation with a transformation efficiency of  $1 \times 10^6$  pfu/10 ng double-stranded cDNA. The library contained approximately  $3 \times 10^6$  independent clones with greater than 95% having inserts with an average size approximating 1.65 kb (range 0.8-2.8 kb). Unamplified library stocks were frozen in glycerol at -70°C until needed. Aliquots of the library were amplified once prior

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to screening by a modification of a solid-state method (Kriegler, M. in *Gene Transfer and Expression: A Laboratory Manual* Stockton Press, NY 1990). Library stocks were titrated on LB plates and then the equivalent of 500-1000 colonies was added to 13 ml of 2 x YT media  
5 containing 0.3% agarose and 100 mg/ml carbenicillin in a 14 ml round-bottom polypropylene tube (Falcon). The bacterial suspension was chilled in a wet ice bath for 1 hour to solidify the suspension, and then grown upright at 37°C for 24 hrs. The resultant bacterial colonies were harvested by centrifugation at 2000 x g at RT for 10 min, resuspended  
10 in 3 ml 2X YT/ carbenicillin. Aliquots were taken for frozen stocks (5%) and plasmid DNA preparation.

#### EXAMPLE 4

##### 1 5 Plasmid DNA Preparation and cRNA Transcription

Plasmid DNA was purified from pellets of solid-state grown bacteria (1000 pools of 500 independent clones each) using the Wizard Miniprep kit according to the manufacturer's instructions (Promega Biotech, Madison, WI). The yield of plasmid DNA from a 14  
20 ml solid-state amplification was 5-10 mg. In preparation for cRNA synthesis, 4 mg of DNA was digested with Not I, and the subsequent linearized DNA was made protein and RNase-free by proteinase K treatment (10 mg for 1 hour at 37°C), followed by two phenol, two chloroform/isoamyl alcohol extractions, and two ethanol precipitations.  
25 The DNA was resuspended in approximately 15 ml of RNase-free water and stored at -70°C until needed. cRNA was synthesized using a kit from Promega Biotech with modifications. Each 50 ml reaction contained: 5 ml of linearized plasmid (approximately 1 mg), 40 mM Tris-HCl (pH=7.5), 6 mM MgCl<sub>2</sub>, 2 mM spermidine, 10 mM NaCl, 10  
30 mM DTT, 0.05 mg/ml bovine serum albumin, 2 units/ml RNasin, 800 mM each of ATP, CTP and UTP, 200 mM GTP, 800 mM m<sup>7</sup>G(5')ppp(5')G, 80 units of T7 RNA polymerase, and approximately 20,000 cpm of <sup>32</sup>P-CTP as a trace for quantitation of synthesized RNA by TCA precipitation. The reaction was incubated for 3 hrs. at 30°C;  
35 20 units of RNase-free DNase was added, and the incubation was

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allowed to proceed for an additional 15 min. at 37°C. cRNA was purified by two phenol, chloroform/isoamyl alcohol extractions, two ethanol precipitations, and resuspended at a concentration of 500 ng/ml in RNase-free water immediately before use.

5

### EXAMPLE 5

#### Aequorin Bioluminescence Assay (ABA) and Clone Identification

The ABA requires injection of library pool cRNA (25  
10 ng/egg for pool sizes of 500 to 10,000) with aequorin cRNA (2 ng/egg)  
supplemented with the G-protein alpha subunit  $G_{\alpha 11}$  (2 ng/egg). To  
facilitate stabilization of synthetic transcripts from aequorin and  $G_{\alpha 11}$   
plasmids, the expression vector pCDNA-3 was modified (termed  
pcDNA-3v2) by insertion (in the *Apa* I restriction enzyme site of the  
15 polylinker) of a cassette to append a poly (A) tract on all cRNA's which  
initiate from the T7 RNA polymerase promoter. This cassette includes  
(5' to 3'): a *Bgl* II site, pA (20) and a *Sfi* I site which can be used for  
plasmid linearization. Polymerase chain reaction (PCR) was utilized to  
generate a DNA fragment corresponding to the open reading frame  
20 (ORF) of the aequorin cDNA with an optimized Kosak translational  
initiation sequence (Inouye, S. *et. al.*, 1985, *Proc. Natl. Acad. Sci. USA*  
82:3154-3158). This DNA was ligated into pcDNA-3v2 linearized with  
*EcoR* I and *Kpn* I in the *EcoR* I/*Kpn* I site of pcDNA-3v2.  $G_{\alpha 11}$  cDNA  
was excised as a *Cla* I/*Not* I fragment from the pCMV-5 vector (Woon,  
25 C. *et. al.*, 1989 *J. Biol. Chem.* 264: 5687-93), made blunt with Klenow  
DNA polymerase and inserted into the *EcoR* V site of pcDNA-3v2.  
cRNA was injected into oocytes using the motorized "Nanoject" injector  
(Drummond Sci. Co., Broomall, PA.) in a volume of 50 nl. Injection  
needles were pulled in a single step using a Flaming/Brown micropipette  
30 puller, Model P-87 (Sutter Instrument Co) and the tips were broken  
using 53X magnification such that an acute angle was generated with the  
outside diameter of the needle being <3 mm. Following injection,  
oocytes were incubated in ND-96 medium, with gentle orbital shaking at  
18°C in the dark. Oocytes were incubated for 24 to 48 hours  
35 (depending on the experiment and the time required for expression of

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the heterologous RNA) before "charging" the expressed aequorin with the essential chromophore coelenterazine. Oocytes were "charged" with coelenterazine by transferring them into 35 mm dishes containing 3 ml charging medium and incubating for 2-3 hours with gentle orbital shaking in the dark at 18°C. The charging medium contained 10 mM coelenterazine (Molecular Probes, Inc., Eugene, OR.) and 30 mM reduced glutathione in OR-2 media (no calcium). Oocytes were then returned to ND-86 medium with calcium medium described above and incubation continued in the dark with orbital shaking until bioluminescence measurements were initiated. Measurement of GHSR expression in oocytes was performed using a Berthold Luminometer LB953 (Wallac Inc., Gaithersburg, MD) connected to a PC running the Autolumat-PC Control software (Wallac Inc., Gaithersburg, MD). Oocytes (singly or in pairs) were transferred to plastic tubes (75 x 12 mm, Sarstedt) containing 2.9 ml  $\text{Ca}^{++}$ -free OR-2 medium. Each cRNA pool was tested using a minimum of 3 tubes containing oocytes. Bioluminescence measurements were triggered by the injection of 0.1 ml of 30 mM MK-677 (1 mM final concentration) and recordings were followed for 2 min. to observe kinetic responses consistent with an  $\text{IP}_3$ -mediated response.

Pool S10-20 was prepared from the unfractionated swine pituitary cDNA library and was composed of 10 pools each of 1000 clones. S10-20 gave a positive signal on two luminometer instruments and the component pools were then individually tested for activity. From the 10 pools of 1000 clones, only pool S271 gave a positive response. This pool was made from two pools of 500 clones designated P541 and P542. Again, only one of the pools, P541, gave a positive bioluminescent signal in the presence of 1 mM Compound A. At this point, the bacterial titer was determined in the glycerol stock of P541 such that dilutions could be plated onto LB agar plates containing 100 mg/ml carbenicillin to yield approximately 50 colonies per plate. A total of 1527 colonies were picked and replicated from 34 plates. The colonies on the original plates were then washed off, plasmids isolated, cRNA synthesized and injected into oocytes. cRNA prepared from 8 of

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- the 34 plates gave positive signals in oocytes. Two plates were selected and the individual colonies from these plates were grown up, plasmid isolated, cRNA prepared and injected into oocytes. A single clonal isolate from each plate (designated as clones 7-3 and 28-18) gave a positive bioluminescence response to 1 mM Compound A. Clone 7-3 was further characterized.

### EXAMPLE 6

#### 1 0 Receptor Characterization

- DNA sequencing was performed on both strands using an automated Applied Biosystems instrument (ABI model 373) and manually by the dideoxy chain termination method using Sequenase II (US Biochemical, Cleveland, OH). Database searches (Genbank 88, EMBL 42, Swiss-Prot 31, PIR 40, dEST, Prosite, dbGPCR ), sequence alignments and analysis of the GHSR nucleotide and protein sequences were carried out using the GCG Sequence Analysis Software Package (Madison, WI; pileup, peptide structure and motif programs), FASTA and BLAST search programs, and the PC/Gene software suite from
- 2 0 Intelligenetics (San Francisco, CA; protein analysis programs). Northern blot analysis was conducted using total (20 mg/lane) or poly (A)+ mRNA (5-10 mg/lane) prepared as described above. RNA was fractionated on a 1% agarose gel containing 2.2 M formaldehyde and blotted to a nitrocellulose membrane. Southern blots were hybridized
- 2 5 with a PCR generated probe encompassing the majority of the ORF predicted by clone 7-3 (nt 291 to 1132). The probe was radiolabeled by random-priming with [a]<sup>32</sup>P-dCTP to a specific activity of greater than 10<sup>9</sup> dpm/mg. Southern blots were pre-hybridized at 42°C for 4 hrs. in 5 X SSC, 5 x Denhardt's solution, 250 mg/ml tRNA, 1% glycine,
- 3 0 0.075% SDS, 50 mM NaPO<sub>4</sub> (pH 6) and 50% formamide. Hybridizations were carried out at 42°C for 20 hrs. in 5 X SSC, 1 X Denhardt's solution, 0.1% SDS, 50 mM NaPO<sub>4</sub>, and 50% formamide. RNA blots were washed in 2 x SSC, 0.2% SDS at 42°C and at -70°C. RNA size markers were 28S and 18S rRNA and *in vitro* transcribed
- 3 5 RNA markers (Novagen). Nylon membranes containing EcoR I and

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Hind III digested genomic DNA from several species (Clontech; 10 mg/lane) were hybridized for 24 hrs. at 30°C in 6 X SSPE, 10 X Denhardt's, 1% SDS, and 50% formamide. Genomic blots were washed twice with room temperature 6 X SSPE, twice with 55°C 6 X SSPE, and  
5 twice with 55°C 4 X SSPE. Additional swine GHSR clones from the swine cDNA library (described above) were identified by hybridization to plasmid DNA (in pools of 500 clones each) immobilized to nylon membranes in a slot-blot apparatus (Scheicher and Schuell). Pure clonal isolates were subsequently identified by colony hybridization. Swine  
10 GHSR clones that extend further in a 5' direction were identified using 5' RACE procedures (Frohman, M. A., 1993 *Methods Enzymol.* 218:340-358, which is incorporated by reference) using swine pituitary poly (A)<sup>+</sup> mRNA as template.

15 EXAMPLE 7

Human GHSR

Human pituitary homologues of the swine GHSR were obtained by screening a commercially available cDNA library  
20 constructed in the vector lambda ZAP II (Stratagene) as per the manufacturer's instructions. Approximately  $1.86 \times 10^6$  phages were initially plated and screened using a random-primer labeled portion of swine clone 7-3 (described above) as hybridization probe. Twenty one positive clones were plaque purified. The inserts from these clones  
25 were excised from the bacteriophage into the phagemid pBluescript II SK- by co-infection with helper phage as described by the manufacturer (Stratagene). Human clones were characterized as has been described above for the swine clone.

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### EXAMPLE 8

#### Assays

- Mammalian cells (COS-7) were transfected with GHSR expression plasmids using Lipofectamine (GIBCO-BRL; Hawley-Nelson, P. 1993, *Focus* 15:73). Transfections were performed in 60 mm dishes on 80% confluent cells (approximately  $4 \times 10^5$  cells) with 8 mg of Lipofectamine and 32 mg of GHSR plasmid DNA.
- Binding of  $^{35}\text{S}$ -Compound A to swine pituitary membranes and crude membranes prepared from COS-7 cells transfected with GHSR expression plasmids was conducted. Crude cell membranes from COS-7 transfectants were prepared on ice, 48 hrs. post-transfection. Each 60 mm dish was washed twice with 3 ml of PBS, once with 1 ml homogenization buffer (50 mM Tris-HCl [pH 7.4], 5 mM  $\text{MgCl}_2$ , 2.5 mM EDTA, 30 mg/ml bacitracin). 0.5 ml of homogenization buffer was added to each dish, cells were removed by scraping and then homogenized using a Polytron device (Brinkmann, Syosset, NY; 3 bursts of 10 sec. at setting 4). The homogenate was then centrifuged for 20 min. at  $11,000 \times g$  at  $0^\circ\text{C}$  and the resulting crude membrane pellet (chiefly containing cell membranes and nuclei) was resuspended in homogenization buffer supplemented with 0.06% BSA (0.1 ml/60 mm dish) and kept on ice. Binding reactions were performed at  $20^\circ\text{C}$  for 1 hr. in a total volume of 0.5 ml containing: 0.1 ml of membrane suspension, 10 ml of  $^{35}\text{S}$ -Compound A (0.05 to 1 nM; specific activity approximately 900 Ci/mmol), 10 ml of competing drug and 380-390 ml of homogenization buffer. Bound radioligand was separated by rapid vacuum filtration (Brandel 48-well cell harvester) through GF/C filters pretreated for 1 hr. with 0.5% polyethylenimine. After application of the membrane suspension to the filter, the filters were washed 3 times with 3 ml each of ice cold 50 mM Tris-HCl [pH 7.4], 10 mM  $\text{MgCl}_2$ , 2.5 mM EDTA and 0.015% Triton X-100, and the bound radioactivity on the filters was quantitated by scintillation counting. Specific binding (> 90% of total) is defined as the difference between total binding and

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non-specific binding conducted in the presence of 50 nM unlabeled Compound A.

### EXAMPLE 9

5

#### Preparation of High Specific Activity Radioligand [ $^{35}\text{S}$ ]-Compound A

[ $^{35}\text{S}$ ]-Compound A was prepared from an appropriate precursor, N-[1(R)-[(1,2-dihydrospiro[3H-indole-3,4'-piperidin]-1'-yl)-carbonyl]-2-(phenyl-methoxy)ethyl]-2-amino-t-butoxycarbonyl-2-methylpropan-amide, using methane [ $^{35}\text{S}$ ]sulfonyl chloride as described in Dean DC, *et al.*, 1995, In: Allen J, Voges R (eds) Synthesis and Applications of Isotopically Labelled Compounds, John Wiley & Sons, New York, pp. 795-801. Purification by semi-preparative HPLC (Zorbax SB-phenyl column, 68% MeOH/water, 0.1% TFA, 5 ml/min) was followed by N-t-BOC cleavage using 15% trifluoro-acetic acid in dichloromethane (25°C, 3 hr) to give [methylsulfonyl- $^{35}\text{S}$ ]Compound A in near quantitative yield. HPLC purification (Hamilton PRP-1 4.6x250 mm column, linear gradient of 50-75% methanol-water with 1 mM HCl over 30 min, 1.3 ml/min) provided the ligand in >99% radiochemical purity. The structure was established by HPLC coelution with unlabeled Compound A and by mass spectral analysis. The latter method also indicated a specific activity of ~1000 Ci/mmol.

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### EXAMPLE 10

25

#### DNA Encoding a Rat Growth Hormone Secretagogue Receptor (GHSR) Type Ia

Cross-hybridization under reduced stringency was the strategy utilized to isolate the rat GHSR type Ia. Approximately  $10^6$  phage plaques of a once-amplified rat pituitary cDNA library in lambda gt11 (RL1051b; Clontech, Palo Alto, CA) were plated on *E. coli* strain Y1090r<sup>-</sup>. The plaques were transferred to maximum-strength Nytran (Schleicher & Schuell, Keene, NH) denatured, neutralized and screened with a 1.6 kb EcoRI/NotI fragment containing the entire coding and

30

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untranslated regions of the swine GHSR, clone 7-3. The membranes were incubated at 30°C in prehybridization solution (50% formamide, 2 X Denhardt's, 5 X SSPE, 0.1% SDS, 100 mg/ml salmon sperm DNA) for 3 hours followed by overnight incubation in hybridization solution (50% formamide, 2 X Denhardt's, 5 X SSPE, 0.1% SDS, 10% dextran sulfate, 100 mg/ml salmon sperm DNA) with  $1 \times 10^6$  cpm/ml of [ $^{32}$ P]-labeled probe. The probe was labeled with [ $^{32}$ P]dCTP using a random priming kit (Gibco BRL, Gaithersburg, ND). After hybridization the blots were washed two times each with 2 X SSC, 0.1% SDS (at 24°C, then 37°C, and finally 55°C). A single positive clone was isolated following three rounds of plaque purification. Phage containing the GHSR was eluted from plate plaques with 1x lambda buffer (0.1M NaCl, 0.01M MgSO<sub>4</sub>·7H<sub>2</sub>O, 35mM Tris-HCl, pH 7.5) following overnight growth of approximately 200 pfu/150mm dish. After a ten minute centrifugation at 10,000 x g to remove debris, the phage solution was treated with 1 mg/ml RNase A and DNase I for thirty minutes at 24°C, followed by precipitation with 20% PEG (8000)/2M NaCl for two hours on ice, and collection by centrifugation at 10,000 x g for twenty minutes. Phage DNA was isolated by incubation in 0.1% SDS, 30mM EDTA, 50 mg/ml proteinase K for one hour at 68°C, with subsequent phenol (three times) and chloroform (twice) extraction before isopropanol precipitation overnight. The GHSR DNA insert (~6.4 kb) was sub-cloned from lambda gt11 into the plasmid vector Litmus 28 (New England Biolabs, Beverly, MA). 2 mg of phage DNA was heated to 65°C for ten minutes, then digested with 100 units BsiWI (New England Biolab, Beverly, MA) at 37°C overnight. A 6.5 kb fragment was gel purified, electroeluted and phenol/chloroform extracted prior to ligation to BsiWI-digested Litmus 28 vector.

Double-stranded DNA was sequenced on both strands on a ABI 373 automated sequencer using the ABI PRISM dye termination cycle sequencing ready reaction kit (Perkin Elmer; Foster City, CA).

Comparison of the complete ORF encoding the rat GHSR type Ia protein sequence to human and swine GHSR homologs reveals a

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high degree of sequence identity (rat vs. human, 95.1 %; rat vs. swine 93.4 %).

For sequence comparisons and functional expression studies, a contiguous DNA fragment encoding the complete ORF (devoid of intervening sequence) for the rat GHSR type Ia was generated. The PCR was utilized to synthesize a amino-terminal fragment from Met-1 to Val-260 with EcoRI (5') and HpaI (3') restriction sites appended, while a carboxyl-terminal fragment was generated from Lys-261 to Thr-364 with Dra I (5') and Not I (3') restriction sites appended. The ORF construct was assembled into the mammalian expression vector pSV7 via a three-way ligation with EcoRI/Not I-digested pSV7, EcoRI/Hpa I-digested NH<sub>2</sub>-terminal fragment, and Dra I/Not I-digested C-terminal fragment.

Functional activity of the ORF construct was assessed by transfecting (using lipofectamine; GIBCO/BRL) 5 mg of plasmid DNA into the aequorin expressing reporter cell line (293-AEQ17) cultured in 60 mm dishes. Following approximately 40 hours of expression the aequorin in the cells was charged for 2 hours with coelenterazine, the cells were harvested, washed and pelleted by low speed centrifugation into luminometer tubes. Functional activity was determined by measuring Compound A dependent mobilization of intracellular calcium and concomitant calcium induced aequorin bioluminescence. Shown in Fig. 26 are three replicate samples exhibiting Compound A-induced luminescent responses.

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WHAT IS CLAIMED IS:

1. A receptor which is a member of the growth hormone family of receptors, free from receptor-associated proteins.  
5
2. Growth hormone secretagogue receptor, free from receptor-associated proteins.
3. A growth hormone secretagogue receptor  
10 according to Claim 2 which is human.
4. A growth hormone secretagogue receptor according to Claim 2 which is from swine.
- 15 5. A growth hormone secretagogue receptor according to Claim 2 which is from rat.
6. Growth hormone secretagogue related receptor, free from receptor-associated proteins.  
20
7. Isolated growth hormone secretagogue receptor.
8. A growth hormone secretagogue receptor according to Claim 7 which is human.  
25
9. A growth hormone secretagogue receptor according to Claim 7 which is from swine.
10. A growth hormone secretagogue receptor  
30 according to Claim 7 which is from rat.
11. A receptor according to Claim 4 or 9 which comprises a full length receptor or which comprises the amino acid sequence as shown in any one of FIGURES 3 or 5.

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12. A receptor according to Claim 3 or 8 which comprises the amino acid sequence as shown in any one of FIGURES 7, 8, 10 or 22.

5 13. A receptor according to Claim 5 or 10 which comprises the amino acid sequence shown in FIGURE 25.

10 14. A functional equivalent of a receptor of Claim 1.

15 15. A functional equivalent of a receptor of Claim 2.

20 16. A functional equivalent of a receptor of Claim 6.

25 17. A nucleic acid which encodes a receptor that is a member of the growth hormone secretagogue family of receptors, said nucleic acid being free from associated nucleic acids.

30 18. A nucleic acid which encodes a growth hormone secretagogue receptor or a functional equivalent, said nucleic acid being free from associated nucleic acids.

35 19. A nucleic acid according to Claim 18 which encodes human growth hormone secretagogue receptor, or a functional equivalent.

40 20. A nucleic acid according to Claim 18 which encodes swine growth hormone secretagogue receptor, or a functional equivalent.

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21. A nucleic acid according to Claim 18 which encodes rat growth hormone secretagogue receptor, or a functional equivalent.

5 22. A nucleic acid according to Claim 17 which encodes a growth hormone secretagogue related to receptor.

23. A nucleic acid according to Claim 18 which is a DNA.

10 24. A nucleic acid according to Claim 23 which is shown in any one of FIGURES 1 or 4.

15 25. A nucleic acid according to Claim 23 which is shown in any one of FIGURES 6, 9 or 11.

26. A nucleic acid according to Claim 23 which is shown in any one of FIGURES 23 or 24.

20 27. A nucleic acid according to Claim 18 which is an RNA.

25 28. A vector comprising a nucleic acid which encodes a receptor which is a member of the growth hormone secretagogue family of receptors.

29. A vector comprising a nucleic acid which encodes a growth hormone secretagogue receptor, or a functional equivalent.

30 30. A vector according to Claim 29 which is selected from the group consisting of: plasmids, modified viruses, yeast artificial chromosomes, bacteriophages, cosmids and transposable elements.

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31. A vector according to Claim 29 wherein the nucleic acid encodes human growth hormone secretagogue receptor or a functional equivalent.

5 32. A vector according to Claim 29 wherein the nucleic acid encodes swine growth hormone secretagogue receptor, or a functional equivalent.

10 33. A vector according to Claim 29 wherein the nucleic acid encodes rat growth hormone secretagogue receptor, or a functional equivalent.

15 34. A vector according to Claim 28 wherein the nucleic acid encodes a growth hormone secretagogue related receptor.

35. A host cell comprising a vector according to Claim 28.

20 36. A host cell comprising a vector according to Claim 28.

25 37. A host cell according to Claim 36 wherein the nucleic acid encodes human growth hormone secretagogue receptor, or a functional equivalent.

38. A host cell according to Claim 36 wherein the nucleic acid encodes swine growth hormone secretagogue receptor, or a functional equivalent.

30 39. A host cell according to Claim 36 wherein the nucleic acid encodes rat growth hormone secretagogue receptor, or a functional equivalent.

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40. A nucleic acid encoding a GPCR clone that belongs to the GHSR family and that hybridizes with a nucleotide which encodes either human, swine or rat GHSR under reduced stringency of hybridization.

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|  |      |      |      |
|--|------|------|------|
| 10   | 20   | 30   | 40   |
| CCTCACGCTGCCAGACCTGGGCTGGGACGCTCCCCCTGAA   | 40   |      |      |
| AACGACTCGCTAGTGGAGGAGCTGCTGCCGCTCTTCCCCA   | 80   |      |      |
| CGCCGCTGTTGGCGGGCGTCACCGCCACCTGCGTGGCGCT   | 120  |      |      |
| CTTCGTGGTGGGTATCGCGGGCAACCTGCTCACGATGCTG   | 160  |      |      |
| GTAGTGTACGCTTCCGCGAGATGCGCACCACCACCAACC    | 200  |      |      |
| 210  | 220  | 230  | 240  |
| TCTACCTGTCCAGCATGGCCTTCTCCGACCTACTCATCTT   | 240  |      |      |
| CCTCTGCATGCCCCCTCGACCTCTTCCGCCCTCTGGCAGTAC | 280  |      |      |
| CGGCCTTGGAAACCTTGGCAACCGCCTCGCAACCTCTTCC   | 320  |      |      |
| AGTTCGTTAGCGAGAGCTGCACCTACGCCACAGTGCACAC   | 360  |      |      |
| CATCACCGCGCGTGGAGCGTGGAGCGCTACTTCGCCATCTCG | 400  |      |      |
| 410  | 420  | 430  | 440  |
| TTCCCGCTGCGGGCCAAGGTAGTGGTCACCAAGGGCCGGG   | 440  |      |      |
| TAAAGCTGGTCATCCTGGTCATCTGGGCCGTGGCCTTCTG   | 480  |      |      |
| CAGCGCCGGGCCATCTTCGTGCTGGTCGGAGTGGAGCAT    | 520  |      |      |
| GATAACGGCACTGACCCTCGGGACACCAACGAGTGGCGCG   | 560  |      |      |
| CCACGGAGTTGCGCGTGGCTCCGGGCTGCTTACCGTCAT    | 600  |      |      |
| 610  | 620  | 630  | 640  |
| GGTCTGGGTGTCCAGTGTCTTCTTCTTCTGCCTGTCTTC    | 640  |      |      |
| TGCCTCACTGTGCTCTATAGCCTCATCGGCAGGAAGCTCT   | 680  |      |      |
| GGCGGAGGAAGCGCGCGGAGGGCGCGGTGGGCTCCTCGCT   | 720  |      |      |
| CAGGGACCAGAACCACAAACAAACCGTGAATATGCTGGCT   | 760  |      |      |
| GTAGTGGTGTGCTTTCATACTCTGCTGGCTGCCTTTCC     | 800  |      |      |
| 810  | 820  | 830  | 840  |
| ATGTAGGGCGATATTTATTTTCCAAATCCTTGGAGCCTGG   | 840  |      |      |
| CTCTGTGGAGATTGCTCAGATCAGCCAATACTGCAACCTC   | 880  |      |      |
| GTGTCCTTTGTCTCTTCTACCTCAGTGGCGCCATCAACC    | 920  |      |      |
| CTATTCTGTACAACATCATGTCCAAGAAGTATCGGGTGGC   | 960  |      |      |
| GGTGTTCAAACTGCTGGGATTGAGCCCTTCTCACAGAGG    | 1000 |      |      |
| 1010                                       | 1020 | 1030 | 1040 |
| AAACTCTCCACTCTGAAGGATGAAAGTTCTCGGGCCTGGA   | 1040 |      |      |
| CAGAATCTAGTATTAAATACATGA                   | 1063 |      |      |

FIG. 1

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|             |             |     |
|-------------|-------------|-----|
| 10          | 20          |     |
| MLVVSREFRM  | RTTTNLYLSS  | 20  |
| MAFSDLLIFL  | CMPLDLFRLW  | 40  |
| QYRPWNLGNL  | LCKLFQFVSE  | 60  |
| SCTYATVLTII | TALSVRYFA   | 80  |
| ICFPLRAKVV  | VTGGRVKI.VI | 100 |
| 110         | 120         |     |
| LVIWAVAFCS  | AGPIFVL.VGV | 120 |
| EHDNGTDPRD  | TNECRATEFA  | 140 |
| VRSGLLTVMV  | WVSSVFFFLP  | 160 |
| VFCLTVLYSL  | IGRKLWRRKR  | 180 |
| GEAAVGSSLR  | DQNHKQTVKM  | 200 |
| 210         | 220         |     |
| LAVVVFAFIL  | CWLPHVGRY   | 220 |
| LFSKSLEPGS  | VEIAQISQYC  | 240 |
| NLVSEVLFYL  | SAAINPILYN  | 260 |
| IMSKKYRVAV  | FKLLGFEPFS  | 280 |
| QRKLSTLKDE  | SSRAWTESSI  | 300 |
| 310         | 320         |     |
| NT          | 302         |     |

FIG.2

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1  
LTLPDLGWD A PPENDSLVEE LLPLFPTPL 30

HELIX 1 60  
AGVTATCVAL FVVG IAGNLL TMLVVS RFRE

HELIX 2 90  
MRITTNLYLS SMAFS DLLIF ICMPLDLFRL

HELIX 3 120  
WQYRPWN LGN LLCKLFQFVS ESCTYATVLT

150  
ITALSVERYF AICFPLRAKV VTKGRVKLV

HELIX 4 180  
ILVIWAVAF C SAGPIFVLVG VEHNGTDPR

210  
DTNECRATEF AVRSGLLTVM VVSSVFFFL

HELIX 5 240  
PVFCLTVLYS LIQRKLWRRK RGEAAVGSSL

HELIX 6 270  
RDQNHKQT VK MLAVVVFAFI LCWLPHVQR

300  
YLFSKSLEPG SVEIAQISQY CNVSVLFY

HELIX 7 330  
LSAAINPILY NIMSKKYRVA VFKLLGFEPF

353  
SQRKLSTLKD ESSRAWTESS INT

FIG.3

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|   |      |      |      |
|---|------|------|------|
| 10  | 20   | 30   | 40   |
| GCAGCCTCTCACTTCCCTCTTTCCTCTCCTAGCATCCTCC  | 40   |      |      |
| CTGAGAGAGCCGCGCTCGATACTCCTTTGCACTCTTTCGCG | 80   |      |      |
| CCTAAGAGAACCTTCTCTGGGACCAGCCGGCTCCACCCTC  | 120  |      |      |
| TCGGTCCTATCCAAGAGCCAGTTAAGCAGAGCCCTAAGCA  | 160  |      |      |
| TGTGGAACGCGACCCCGAGCGAGGAACCGGGGCCAACCT   | 200  |      |      |
| 210                                       | 220  | 230  | 240  |
| CACGCTGCCAGACCTGGGCTGGGACGCTCCCCGTGAAAAC  | 240  |      |      |
| GACTCGCTAGTGGAGGAGCTGCTGCCGCTCTTCCCCACGC  | 280  |      |      |
| CGCTGTTGGCGGGCGTCACCGCCACCTGCGTGGCGCTCTT  | 320  |      |      |
| CGTGGTGGGTATCGCGGGCAACCTGCTCACGATGCTGGTA  | 360  |      |      |
| GTGTACGCTTCCGCGAGATGCGCACCAACCAACCTCT     | 400  |      |      |
| 410                                       | 420  | 430  | 440  |
| ACCTGTCCAGCATGGCCTTCTCCGACCTACTCATCTTCCT  | 440  |      |      |
| CTGCATGCCCCCTCGACCTCTTCGCCTTTGGCAGTACCGG  | 480  |      |      |
| CCTTGAACCTTGGCAACCTGCTCTGCAAACTCTTCCAGT   | 520  |      |      |
| TCGTTAGCGAGAGCTGCACCTACGCCACAGTGCTCACCAI  | 560  |      |      |
| CACCGCGCTGAGCGTCGAGCGCTACTTCGCCATCTGCTTC  | 600  |      |      |
| 610                                       | 620  | 630  | 640  |
| CCGCTGCGGGCCAAGGTAGTGGTCACCAAGGGCCGGGTAA  | 640  |      |      |
| AGCTGGTCATCCTGGTCATCTGGGCCGTGGCCTTCTGCAG  | 680  |      |      |
| CGCCGGGCCCATCTTCGTGCTGGTGGAGTGGAGCATGAT   | 720  |      |      |
| AACGGCACTGACCCTCGGGACACCAACGAGTGCCGCGCCA  | 760  |      |      |
| CGGAGTTGCGCGTGCGCTCCGGGCTGCTTACCGTCATGGT  | 800  |      |      |
| 810                                       | 820  | 830  | 840  |
| CTGGGTGTCCAGTGCTTCTTCTTCCTGCCTGTCTTCTGC   | 840  |      |      |
| CTCACTGTGCTCTATAGCCTCATCGGCAGGAAGCTCTGGC  | 880  |      |      |
| GGAGGAAGCGCGCGAGGCGGCGTGGGCTCCTCGCTCAG    | 920  |      |      |
| GGACCAGAACCAAAACCAACCGTGAATGCTGGGTGGG     | 960  |      |      |
| TCTCAATGCGCCCTCGAGCTTCTCTCCGGGTCCCCTC     | 1000 |      |      |
| 1010                                      | 1020 | 1030 | 1040 |
| ACTCCTCGTGCCCTTTCTCTTCTCCCTGA             | 1029 |      |      |

FIG.4

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|            |            |            |            |     |
|------------|------------|------------|------------|-----|
| 10         | 20         | 30         | 40         |     |
| MWNATPSEEP | GPNTLPDLG  | WDAPPENDSL | VEELLPLFPT | 40  |
| PLLAGVTATC | VALFVVGIAG | NLTMLVVS   | FREMRITTNL | 80  |
| YLSSMAFSDL | LIFLCMPLDL | FRLWQYRPWN | LGNLLCKLFQ | 120 |
| FVSESCYAT  | VLITALSVE  | RYFAICFPLR | AKVVVTKGRV | 160 |
| KLVILVIWAV | AFCAGPIFV  | LVGVEHDNGT | DPRDTNECRA | 200 |
| 210        | 220        | 230        | 240        |     |
| TEFAVRSGLL | TVMVWSSVF  | FFLPVFCLTV | LYSLIGRKLW | 240 |
| RRKRGEAAVG | SSLRDQNHKQ | TVKMLGGSQC | ALELSLPGPL | 280 |
| HSSCLFSSP  | 289        |            |            |     |

FIG.5

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| 10  | 20   | 30   | 40   |      |
|---|------|------|------|------|
| CGCCCAGCGAAGAGCCGGGTTCAACCTCACACTGGCCGA   |      |      |      | 40   |
| CCTGGACTGGGATGCTTCCCCGGCAACGACTCGCTGGGC   |      |      |      | 80   |
| GACGAGCTGCTGCAGCTCTTCCCCGCGCCGCTGCTGGCGG  |      |      |      | 120  |
| GCGTCACAGCCACCTGCGTGGCACTCTTCGTGGTGGGTAT  |      |      |      | 160  |
| CGCTGGCAACCTGCTCACCATGCTGGTGGTGTGCGGCTTC  |      |      |      | 200  |
| 210                                       | 220  | 230  | 240  |      |
| CGCGAGCTGCGCACCACCACCAACCTCTACCTGTCCAGCA  |      |      |      | 240  |
| TGGCCTTCTCCGATCTGCTCATCTTCCTCTGCATGCCCT   |      |      |      | 280  |
| GGACCTCGTTCGCCCTCTGGCAGTACCGGCCCTGGAATTTC |      |      |      | 320  |
| GGCGACCTCCTCTGCAAACTCTTCCAATTCGTAGTGAGA   |      |      |      | 360  |
| GCTGCACCTACGCCACGGTGCTCACCATCACAGCGCTGAG  |      |      |      | 400  |
| 410                                       | 420  | 430  | 440  |      |
| CGTCGAGCGCTACTTCGCCATCTGCTTCCCACTCCGGGGCC |      |      |      | 440  |
| AAGGTGGTGGTCACCAAGGGGCGGGTGAAGCTGGTCATCT  |      |      |      | 480  |
| TCGTATCTGGGCCGTGGCCTTCTGCAGCGCCGGGCCCAT   |      |      |      | 520  |
| CTTCGTGCTAGTCGGGGTGGAGCAGGAAACGGCACCGAC   |      |      |      | 560  |
| CCTTGGGACACCAACGAGTGCCGCCCCACCGAGTTTGCGG  |      |      |      | 600  |
| 610                                       | 620  | 630  | 640  |      |
| TGCGCTCTGGACTGCTCACGGTCATGGTGTGGGTGTCCAG  |      |      |      | 640  |
| CATCTTCTTCTTCCCTTCTGTCTTCTGTCTCACGGTCCTC  |      |      |      | 680  |
| TACAGTCTCATCGGCAGGAAGCTGTGGCGGAGGAGGCGCG  |      |      |      | 720  |
| GCGATGCTGTCTGGGTGCCTCGCTCAGGGACCAGAAACCA  |      |      |      | 760  |
| CAAGCAAACCGTGAAAATGCTGGCTGTAGTGGTGTGTTGCC |      |      |      | 800  |
| 810                                       | 820  | 830  | 840  |      |
| TTCATCCTCTGCTGGCTCCCCTTCCACGTAGGGCGATATT  |      |      |      | 840  |
| TATTTTCAAATCCTTTGAGCCTGGCTCCTTGGAGATTGC   |      |      |      | 880  |
| TCAGATCAGCCAGTACTGCAACCTCGTGTCTTTGTCTC    |      |      |      | 920  |
| TTCTACCTCAGTGCTGCCATCAACCCATTCTGTACAACA   |      |      |      | 960  |
| TCATGTCCAAGAAGTACCGGGTGGCAGTGTTCAGACTTCI  |      |      |      | 1000 |
| 1010                                      | 1020 | 1030 | 1040 |      |
| GGGATTGCAACCCTTCTCCCAGAGAAAGCTCTCCACTCTG  |      |      |      | 1040 |
| AAAGATGAAAGTTCTCGGGCCTGGACAGAATCTAGTATTA  |      |      |      | 1080 |
| ATACATGA                                  |      |      |      | 1088 |

FIG.6

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| 10          | 20         |     |
|-------------|------------|-----|
| MLVVSRLFEL  | RTTTNLYLSS | 20  |
| MAFSDLLIFL  | CMPLDLVRLW | 40  |
| QYRPWNFGDL  | LCKLFQFVSE | 60  |
| SCTYATVLT I | TALSVERYFA | 80  |
| ICFPLRAKVV  | VTKGRVKLVI | 100 |

| 110        | 120        |     |
|------------|------------|-----|
| FVIWAVAFCS | AGPIFVLVGV | 120 |
| EHENGDPWD  | TNECRPTEFA | 140 |
| VRSGLLTVMV | WVSSIFFFLP | 160 |
| VFCLTVLYSL | IGRKLWRRRR | 180 |
| GDAVVGASLR | DQNHKQTVKM | 200 |

| 210         | 220         |     |
|-------------|-------------|-----|
| LAVVVFAFIL  | CWLPHVGVRY  | 220 |
| LFSKSFEPGS  | LEIAQISQYC  | 240 |
| NLVSFVL FYL | SAAINPILYN  | 260 |
| IMSKKYRVAV  | FRL LGFEPFS | 280 |
| QRKLSTLKDE  | SSRAWTESSI  | 300 |

| 310 | 320 |  |
|-----|-----|--|
| NT  | 302 |  |

FIG.7

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1 30  
PSEEPGFNLT LADLDWDASP GNDSLGDELL

HELIX 1 60  
QLFPAPLAG VTATCVLFV VGIAGNLLTM

HELIX 2 90  
LVVSRFRELR TTTNLYLSSM AFSDLLIFLC

120  
MPLDLVRLWQ YRPWNFGDLL CKLFQFVSES

HELIX 3 150  
CTYATVLTIT ALSVERYTAI CFPLRAKVVV

HELIX 4 180  
TKGRVKLVIF VIWAVAFCSA GPIFVLVGVE

210  
HENGTDPWDT NECRPTEFAV RSGLLTVMVW

HELIX 5 240  
VSSIFFFLPV FCLTVLYSLI QRKLWRRRRG

HELIX 6 270  
DAVVGASLRD QNHKQTVKML AVVVFAFILC

300  
WLPFHVGRYL FSKSFEPGSL EIAQISQYCN

HELIX 7 330  
LVSFVLFYLS AAINPILYNI MSKKYRVAVF

360  
RLLGFEFESQ RKLSTLKDES SRAWTESSIN

361  
T

FIG.8

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|   |     |     |     |      |
|---|-----|-----|-----|------|
| 10  | 20  | 30  | 40  |      |
| GCGCCTCAGCTCCCGCTTCGCGGCGCCTGGTCCCTGCGG   |     |     |     | 40   |
| TCCCCACTCGCTGCGACGCTTTGGGAAGTGGGAGATGGAA  |     |     |     | 80   |
| CTGGATCGAGAACGCAATGCGAGGCAGGGCTGGTGACAG   |     |     |     | 120  |
| CATCCTCCCTACGCGTCTGCACCCGCTCCTCCCTCGCACC  |     |     |     | 160  |
| CTCCCGCGCCTAAGCGGACCTCCTCGGGAGCCAGCTCGGT  |     |     |     | 200  |
| 210                                       | 220 | 230 | 240 |      |
| CCAGCCTCCCAGCGCAGTCACGTCCCAGAGCCTGTTTCAGC |     |     |     | 240  |
| TGAGCCGGCAGCATGTGGAACGCGACGCCAGCGAAGAGC   |     |     |     | 280  |
| CGGGGTTCAACCTCACACTGGCCGACCTGGACTGGGATGC  |     |     |     | 320  |
| TTCCCCCGGCAACGACTCGCTGGGCGACGAGCTGCTGCAG  |     |     |     | 360  |
| CTCTTCCCCCGCGCGCTGCTGGCGGGCGTCAAGCCACCT   |     |     |     | 400  |
| 410                                       | 420 | 430 | 440 |      |
| GCGTGGCACTCTTCGTGGTGGGTATCGCTGGCAACCTGCT  |     |     |     | 440  |
| CACCATGCTGGTGGTGTGCGCTTCCGCGAGCTGCGCACC   |     |     |     | 480  |
| ACCACCAACCTCTACCTGTCCAGCATGGCCTTCTCCGATC  |     |     |     | 520  |
| TGCTCATCTTCTCTGCATGCCCCTGGACCTCGTTGCGCT   |     |     |     | 560  |
| CTGGCAGTACCGGCCCTGGAACCTCGGCGACCTCCTCTGC  |     |     |     | 600  |
| 610                                       | 620 | 630 | 640 |      |
| AAACTCTTCCAATTCGTCACTGAGAGCTGCACCTACGCCA  |     |     |     | 640  |
| CGGTGCTCACCATCACAGCGCTGAGCGTCGAGCGCTACTT  |     |     |     | 680  |
| CGCCATCTGCTTCCCACTCCGGGCCAAGGTGGTGGTCACC  |     |     |     | 720  |
| AAGGGGCGGGTGAAGCTGGTCATCTTCGTCATCTGGGCCG  |     |     |     | 760  |
| TGGCCTTCTGCAGCGCCGGGCCATCTTCGTGCTAGTCGG   |     |     |     | 800  |
| 810                                       | 820 | 830 | 840 |      |
| GGTGGAGCACGAGAACGGCACCGACCCTTGGGACACCAAC  |     |     |     | 840  |
| GAGTGCCGCCCCACCGAGTTTGCGGTGCGCTCTGGACTGC  |     |     |     | 880  |
| TCACGGTCATGGTGTGGGTGTCCAGCATCTTCTTCTTCT   |     |     |     | 920  |
| TCCTGTCTTCTGTCTCACGGTCCTCTACAGTCTCATCGGC  |     |     |     | 960  |
| AGGAAGCTGTGGCGGAGGAGGCGCGCGATGCTGTGCTGG   |     |     |     | 1000 |

FIG.9A

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| 1010                                     | 1020 | 1030 | 1040 |      |
|--|------|------|------|------|
| GTGCCTCGCTCAGGGACCAGAACCACAAGCAAACCGTGAA |      |      |      | 1040 |
| AATGCTGGGTGGGTCTCAGCGCGCGCTCAGGCTTTCTCTC |      |      |      | 1080 |
| GCGGGTCCTATCCTCTCCCTGTGCCTTCTCCCTTCTCTC  |      |      |      | 1120 |
| GA                                       |      |      |      | 1122 |

FIG.9B

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| 10  | 20  | 30  | 40  |
|---|-----|-----|-----|
| MWNATPSEEPGFNLTLADLDWDASPGNDSLGDELLQLFPA      |     |     | 40  |
| PLLAGVTATCVALFVVGIAGNLLTMLVVSRLFELRTTTNL      |     |     | 80  |
| YLSSMAFSDLLIFLCMPDLVRLWQYRPWNFGDLLCKLFQ       |     |     | 120 |
| FVSESCYATVLTITALSVERYFAICFPLRAKVVTGKRV        |     |     | 160 |
| KLVI FVIWAVAFCSAGPIFVLVGVEHNGTDPWDTNECRP      |     |     | 200 |
| 210   | 220 | 230 | 240 |
| TEFAVRSGLLTVMVWSSIFFFLPVFCLTVLYSLIGRKLW       |     |     | 240 |
| RRRRGDAVVGASLRDQNIHKQTVKMI GGSQRAI RI SI AGPI |     |     | 280 |
| LSLCLLPSL                                     |     |     | 289 |

FIG.10

| 10                                       | 20  | 30  | 40  |
|--|-----|-----|-----|
| MPLDLVRLWQYRPWNFGDLLCKLFQFVSESCYATVLTIT  |     |     | 40  |
| ALSVRYFAICFPLRAKVVTGKRVKLVI FVIWAVAFCSA  |     |     | 80  |
| GPIFVLVGVEHNGTDPWDTNECRPTEFAVRSGLLTVMVW  |     |     | 120 |
| VSSIFFFLPVFCLTVLYSLIGRKLWRRRRGDAVVGASLRD |     |     | 160 |
| QNHKQTVKMLAVVVFALCWLPFHVGRYLFKSFEPGSL    |     |     | 200 |
| 210                                      | 220 | 230 | 240 |
| EIAQISQYCNLVSFVLFYLSAAINPILYNIMSKKYRVAVF |     |     | 240 |
| RLLGFEPFSQRKLSTLKDESSRAWTESSJNT          |     |     | 271 |

FIG.12

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| 10                                       | 20  | 30  | 40  |
|--|-----|-----|-----|
| ATCTGCTCATCTTCCTCTGCATGCCCTGGACCTCGTTCC  |     |     | 40  |
| CCTCTGGCAGTACCGGCCCTGGAACCTCGGCGACCTCCTC |     |     | 80  |
| TGCAAACTCTTCCAATTCGTCACTGAGAGCTGCACCTACG |     |     | 120 |
| CCACGGTGCTACCATCACAGCGCTGAGCGTCGAGCGCTA  |     |     | 160 |
| CTTCGCCATCTGCTTCCCACTCCGGGCCAAGGTGGTGGTC |     |     | 200 |
| 210                                      | 220 | 230 | 240 |
| ACCAAGGGGCGGGTGAAGCTGGTCATCTTCGTCACTGGG  |     |     | 240 |
| CCGTGGCCTTCTGCAGCGCCGGGCCCATCTTCGTGCTAGT |     |     | 280 |
| CGGGGTGGAGCACGAGAACGGCACCGACCTTGGGACACC  |     |     | 320 |
| AACGAGTGCCGCCCCACGAGTTTGCGGTGCGCTCTGGAC  |     |     | 360 |
| TGCTCACGGTCATGGTGTGGGTGTCCAGCATCTTCTTCTT |     |     | 400 |
| 410                                      | 420 | 430 | 440 |
| CCTTCCTGTCTTCTGTCTCACGGTCCTCTACAGTCTCATC |     |     | 440 |
| GGCAGGAAGCTGTGGCGGAGGAGCGCGCGGATGCTGTCC  |     |     | 480 |
| TGGGTGCCTCGCTCAGGGACCAGAACCAAGCAAACCGT   |     |     | 520 |
| GAAAATGCTGGCTGTAGTGGTGTTCCTTCATCCTCTGC   |     |     | 560 |
| TGGCTCCCCTTCCACGTAGGGCGATATTTATTTCCAAAT  |     |     | 600 |
| 610                                      | 620 | 630 | 640 |
| CCTTTGAGCCTGGCTCCTTGGAGATTGCTCAGATCAGCCA |     |     | 640 |
| GTA CTGCAACCTCGTGTCTTTGTCTCTTCTACCTCAGT  |     |     | 680 |
| GCTGCCATCAACCCATTCTGTACAACATCATGTCCAAGA  |     |     | 720 |
| AGTACCGGGTGGCAGTGTTTCACTTCTGGGATTCGAACC  |     |     | 760 |
| CTTCTCCAGAGAAAGCTCTCCACTCTGAAAGATGAAAGT  |     |     | 800 |
| 810                                      | 820 | 830 | 840 |
| TCTCGGGCCTGGACAGAACTAGTATTAAATACATGA     |     |     | 836 |

FIG. 11

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|                                 |                        |      |
|---------------------------------|------------------------|------|
| FIG.3-SWINE TYPE I CLONE 7-3orf | v10                    | v20  |
|                                 | LTL.PDLGWDAPPENDSLVEE  |      |
| FIG.5-SWINE TYPE II CLONE 1375m | LTL.PDLGWDAPPENDSLVEE  |      |
|                                 | ^20                    | ^30  |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v30                    | v40  |
|                                 | LLPLFPTPLLAGVTATCVAL   |      |
| FIG.5-SWINE TYPE II CLONE 1375m | LLPLFPTPLLAGVTATCVAL   |      |
|                                 | ^40                    | ^50  |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v50                    | v60  |
|                                 | FVVGIAGNI.ITMI.VVSRFRI |      |
| FIG.5-SWINE TYPE II CLONE 1375m | FVVGIAGNLLTML.VVSRFRE  |      |
|                                 | ^60                    | ^70  |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v70                    | v80  |
|                                 | MRTTTNLYLSSMAFSDLLIF   |      |
| FIG.5-SWINE TYPE II CLONE 1375m | MRTTTNLYLSSMAFSDLLIF   |      |
|                                 | ^80                    | ^90  |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v90                    | v100 |
|                                 | LCMPLDLFRLWQYRPWNLGN   |      |
| FIG.5-SWINE TYPE II CLONE 1375m | LCMPLDLFRLWQYRPWNLGN   |      |
|                                 | ^100                   | ^110 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v110                   | v120 |
|                                 | LLCKLFQFVSESCYATVLT    |      |
| FIG.5-SWINE TYPE II CLONE 1375m | LLCKLFQFVSESCYATVLT    |      |
|                                 | ^120                   | ^130 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v130                   | v140 |
|                                 | ITALSVERYFAICFPLRAKV   |      |
| FIG.5-SWINE TYPE II CLONE 1375m | ITALSVERYFAICFPLRAKV   |      |
|                                 | ^140                   | ^150 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v150                   | v160 |
|                                 | VVTKGRVKLVILVIWAVAF    |      |
| FIG.5-SWINE TYPE II CLONE 1375m | VVTKGRVKLVILVIWAVAF    |      |
|                                 | ^160                   | ^170 |

FIG.13A

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|                                 |                      |      |
|---------------------------------|----------------------|------|
|                                 | v170                 | v180 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | SAGPIFVLVGVEHDNGTDPR |      |
|                                 | SAGPIFVLVGVEHDNGTDPR |      |
| FIG.5-SWINE TYPE II CLONE 1375m | SAGPIFVLVGVEHDNGTDPR |      |
|                                 | ^180                 | ^190 |
|                                 | v190                 | v200 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | DTNECRATEFAVRSGLLTVM |      |
|                                 | DTNECRATEFAVRSGLLTVM |      |
| FIG.5-SWINE TYPE II CLONE 1375m | DTNECRATEFAVRSGLLTVM |      |
|                                 | ^200                 | ^210 |
|                                 | v210                 | v220 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | VWVSSVFFFLPVFCLTVLYS |      |
|                                 | VWVSSVFFFLPVFCLTVLYS |      |
| FIG.5-SWINE TYPE II CLONE 1375m | VWVSSVFFFLPVFCLTVLYS |      |
|                                 | ^220                 | ^230 |
|                                 | v230                 | v240 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | LIGRKLRRRKRGEAAVGSSL |      |
|                                 | LIGRKLRRRKRGEAAVGSSL |      |
| FIG.5-SWINE TYPE II CLONE 1375m | LIGRKLRRRKRGEAAVGSSL |      |
|                                 | ^240                 | ^250 |
|                                 | v250                 | v260 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | RDQNIHQTVKMLAVVVFAFI |      |
|                                 | RDQNHQTVKML: A:      |      |
| FIG.5-SWINE TYPE II CLONE 1375m | RDQNHQTVKMLGGSQCALE  |      |
|                                 | ^260                 | ^270 |
|                                 | v270                 |      |
| FIG.3-SWINE TYPE I CLONE 7-3orf | LCWL-PFHVGRYLFSSKS   |      |
|                                 | L...P:H...LFS..      |      |
| FIG.5-SWINE TYPE II CLONE 1375m | LSLPGPLH-SSCLFSSP    |      |
|                                 | ^280                 |      |

FIG.13B

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|                                 |   |
|---------------------------------|---|
| FIG.8-HUMAN TYPE I 1146orf      | v10 v20<br>PSEEPGFNLTADLDWDASP<br>PSEEPGFNLTADLDWDASP     |
| FIG.10-HUMAN TYPE II CLONE1141m | ^10 ^20<br>PSEEPGFNLTADLDWDASP                            |
| FIG.8-HUMAN TYPE I 1146orf      | v30 v40<br>GNDSLGDPELLQLFPAPLLAG<br>GNDSLGDPELLQLFPAPLLAG |
| FIG.10-HUMAN TYPE II CLONE1141m | ^30 ^40<br>GNDSLGDPELLQLFPAPLLAG                          |
| FIG.8-HUMAN TYPE I 1146orf      | v50 v60<br>VTATCVALFVVGIAGNLTMT<br>VTATCVALFVVGIAGNLTMT   |
| FIG.10-HUMAN TYPE II CLONE1141m | ^50 ^60<br>VTATCVALFVVGIAGNLTMT                           |
| FIG.8-HUMAN TYPE I 1146orf      | v70 v80<br>LVVSRFRELRTTTNLYLSSM<br>LVVSRFRELRTTTNLYLSSM   |
| FIG.10-HUMAN TYPE II CLONE1141m | ^70 ^80<br>LVVSRFRELRTTTNLYLSSM                           |
| FIG.8-HUMAN TYPE I 1146orf      | v90 v100<br>AFSDLLIFLCMPDLVRLWQ<br>AFSDLLIFLCMPDLVRLWQ    |
| FIG.10-HUMAN TYPE II CLONE1141m | ^90 ^100<br>AFSDLLIFLCMPDLVRLWQ                           |
| FIG.8-HUMAN TYPE I 1146orf      | v110 v120<br>YRPWNFGDLLCKLFQFVSES<br>YRPWNFGDLLCKLFQFVSES |
| FIG.10-HUMAN TYPE II CLONE1141m | ^100 ^110<br>YRPWNFGDLLCKLFQFVSES                         |
| FIG.8-HUMAN TYPE I 1146orf      | v130 v140<br>CTYATVLTITALSVERYFAI<br>CTYATVLTITALSVERYFAI |
| FIG.10-HUMAN TYPE II CLONE1141m | ^130 ^140<br>CTYATVLTITALSVERYFAI                         |
| FIG.8-HUMAN TYPE I 1146orf      | v150 v160<br>CFPLRAKVVTGKRVKLVIF<br>CFPLRAKVVTGKRVKLVIF   |
| FIG.10-HUMAN TYPE II CLONE1141m | ^150 ^160<br>CFPLRAKVVTGKRVKLVIF                          |

FIG.14A

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|                                 |                      |
|---------------------------------|----------------------|
| FIG.8-HUMAN TYPE I 1146orf      | v170      v180       |
|                                 | VIWAVAFCSAGPIFVLVGVE |
| FIG.10-HUMAN TYPE II CLONE1141m | VIWAVAFCSAGPIFVLVGVE |
|                                 | ^170      ^180       |
| FIG.8-HUMAN TYPE I 1146orf      | v190      v200       |
|                                 | HENGTDPWDTNECRPTEFAV |
| FIG.10-HUMAN TYPE II CLONE1141m | HENGTDPWDTNECRPTEFAV |
|                                 | ^190      ^200       |
| FIG.8-HUMAN TYPE I 1146orf      | v210      v220       |
|                                 | RSGLLTVMVWVSSIFFFLPV |
| FIG.10-HUMAN TYPE II CLONE1141m | RSGLLTVMVWVSSIFFFLPV |
|                                 | ^210      ^220       |
| FIG.8-HUMAN TYPE I 1146orf      | v230      v240       |
|                                 | FCLTVLYSLIGRKLWRRRRG |
| FIG.10-HUMAN TYPE II CLONE1141m | FCLTVLYSLIGRKLWRRRRG |
|                                 | ^230      ^240       |
| FIG.8-HUMAN TYPE I 1146orf      | v250      v260       |
|                                 | DAVVGASLRDQNHKQTVKML |
| FIG.10-HUMAN TYPE II CLONE1141m | DAVVGASLRDQNHKQTVKML |
|                                 | ^250      ^260       |

FIG.14B

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|                                 |   |      |      |      |
|---------------------------------|---|------|------|------|
| FIG.3-SWINE TYPE I CLONE 7-3orf | v10                                       | v20  | v30  | v40  |
|                                 | LTLPLDGLWDAPPENDSLVEELLPLFPTPLLAGVTATCVAL |      |      |      |
| FIG.8-HUMAN TYPE I 1146orf      | LTL:DL:WDA:P:NDSL:ELL:LFP:PLLAGVTATCVAL   |      |      |      |
|                                 | ^10                                       | ^20  | ^30  | ^40  |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v50                                       | v60  | v70  | v80  |
|                                 | FVVGIAGNLLTMLVVSFRFEMRTTTNLYLSSMAFSDLLIF  |      |      |      |
| FIG.8-HUMAN TYPE I 1146orf      | FVVGIAGNLLTMLVVSFRF:RTTTNLYLSSMAFSDLLIF   |      |      |      |
|                                 | ^50                                       | ^60  | ^70  | ^80  |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v90                                       | v100 | v110 | v120 |
|                                 | LCMPDLDFRLWQYRPWNLGILLCKLFQFVSESCYATVLT   |      |      |      |
| FIG.8-HUMAN TYPE I 1146orf      | LCMPDLRLWQYRPWN:G:LLCKLFQFVSESCYATVLT     |      |      |      |
|                                 | ^90                                       | ^100 | ^110 | ^120 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v130                                      | v140 | v150 | v160 |
|                                 | ITALSVRYFAICFPLRAKVVTGKRVKLVILVIWAVAF     |      |      |      |
| FIG.8-HUMAN TYPE I 1146orf      | ITALSVRYFAICFPLRAKVVTGKRVKLV:VIWAVAF      |      |      |      |
|                                 | ^130                                      | ^140 | ^150 | ^160 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v170                                      | v180 | v190 | v200 |
|                                 | SAGPIFVLVGVEHDNGTDPDRTNECRATEFAVRSGLLTVM  |      |      |      |
| FIG.8-HUMAN TYPE I 1146orf      | SAGPIFVLVGVEH:NGTDP:DTNECR:TEFAVRSGLLTVM  |      |      |      |
|                                 | ^170                                      | ^180 | ^190 | ^200 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v210                                      | v220 | v230 | v240 |
|                                 | VWVSSVFFFLPVFCLTVLYSLIGRKLWRRKRGEAAVGSSL  |      |      |      |
| FIG.8-HUMAN TYPE I 1146orf      | VWVSS:FFFLPVFVLTLYSLIGRKLWRR:RG:A:VG:SL   |      |      |      |
|                                 | ^210                                      | ^220 | ^230 | ^240 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v250                                      | v260 | v270 | v280 |
|                                 | RDQNHKQTVKMLAVVVFAFILCWLPFHVGRYLFSKSLFPG  |      |      |      |
| FIG.8-HUMAN TYPE I 1146orf      | RDQNHKQTVKMLAVVVFAFILCWLPFHVGRYLFSK:EPG   |      |      |      |
|                                 | ^250                                      | ^260 | ^270 | ^280 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v290                                      | v300 | v310 | v320 |
|                                 | SVEIAQISQYCNLVSFVLYLSAAINPILYNIMSKKYRVA   |      |      |      |
| FIG.8-HUMAN TYPE I 1146orf      | S:EIAQISQYCNLVSFVLYLSAAINPILYNIMSKKYRVA   |      |      |      |
|                                 | ^290                                      | ^300 | ^310 | ^320 |
| FIG.3-SWINE TYPE I CLONE 7-3orf | v330                                      | v340 | v350 |      |
|                                 | VFKLLGFEPFSQKRLSTLKDESSRAWTESSINT         |      |      |      |
| FIG.8-HUMAN TYPE I 1146orf      | VF:LLGFEPFSQKRLSTLKDESSRAWTESSINT         |      |      |      |
|                                 | ^330                                      | ^340 | ^350 | ^360 |

FIG.15

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|                                 |                       |      |
|---------------------------------|-----------------------|------|
| FIG.5-SWINE TYPE II CLONE 1375m | v10                   | v20  |
|                                 | MWNATPSEEPGNLTLPDLG   |      |
| FIG.10-HUMAN TYPE II CLONE1141m | MWNATPSEEPG NLTL:DL:  |      |
|                                 | MWNATPSEEPGNLTADLD    |      |
|                                 | ^10                   | ^20  |
|                                 | v30                   | v40  |
| FIG.5-SWINE TYPE II CLONE 1375m | WDAPPENDSLVEELLPLFPT  |      |
| FIG.10-HUMAN TYPE II CLONE1141m | WDA:P.NDSL :ELL.LFP:  |      |
|                                 | WDASPGNDSLGLDELLQLFPA |      |
|                                 | ^30                   | ^40  |
|                                 | v50                   | v60  |
| FIG.5-SWINE TYPE II CLONE 1375m | PLLAGVTATCVAI FVVGIAG |      |
| FIG.10-HUMAN TYPE II CLONE1141m | PLLAGVTATCVAI FVVGIAG |      |
|                                 | PLLAGVTATCVALFVVGIAG  |      |
|                                 | ^50                   | ^60  |
|                                 | v70                   | v80  |
| FIG.5-SWINE TYPE II CLONE 1375m | NLLTMLVVSFRFEMRTTTL   |      |
| FIG.10-HUMAN TYPE II CLONE1141m | NLLIMLVVSFRF:RITTL    |      |
|                                 | NLLTMLVVSFRFRELRTTTL  |      |
|                                 | ^70                   | ^80  |
|                                 | v90                   | v100 |
| FIG.5-SWINE TYPE II CLONE 1375m | YLSSMAFSDDLIFLCMPDL   |      |
| FIG.10-HUMAN TYPE II CLONE1141m | YLSSMAFSDDLIFLCMPDL   |      |
|                                 | YLSSMAFSDDLIFLCMPDL   |      |
|                                 | ^90                   | ^100 |
|                                 | v110                  | v120 |
| FIG.5-SWINE TYPE II CLONE 1375m | FRLWQYRPWNLGNLLCKLFQ  |      |
| FIG.10-HUMAN TYPE II CLONE1141m | RLWQYRPWN:G:LLCKLFQ   |      |
|                                 | VRLWQYRPWNFGDLLCKLFQ  |      |
|                                 | ^110                  | ^120 |
|                                 | v130                  | v140 |
| FIG.5-SWINE TYPE II CLONE 1375m | FVSECTYATVLTITALSVE   |      |
| FIG.10-HUMAN TYPE II CLONE1141m | FVSECTYATVLTITALSVE   |      |
|                                 | FVSECTYATVLTITALSVE   |      |
|                                 | ^130                  | ^140 |
|                                 | v150                  | v160 |
| FIG.5-SWINE TYPE II CLONE 1375m | RYFAICFPLRAKVVVTKGRV  |      |
| FIG.10-HUMAN TYPE II CLONE1141m | RYFAICFPLRAKVVVTKGRV  |      |
|                                 | RYFAICFPLRAKVVVTKGRV  |      |
|                                 | ^150                  | ^160 |

FIG.16A

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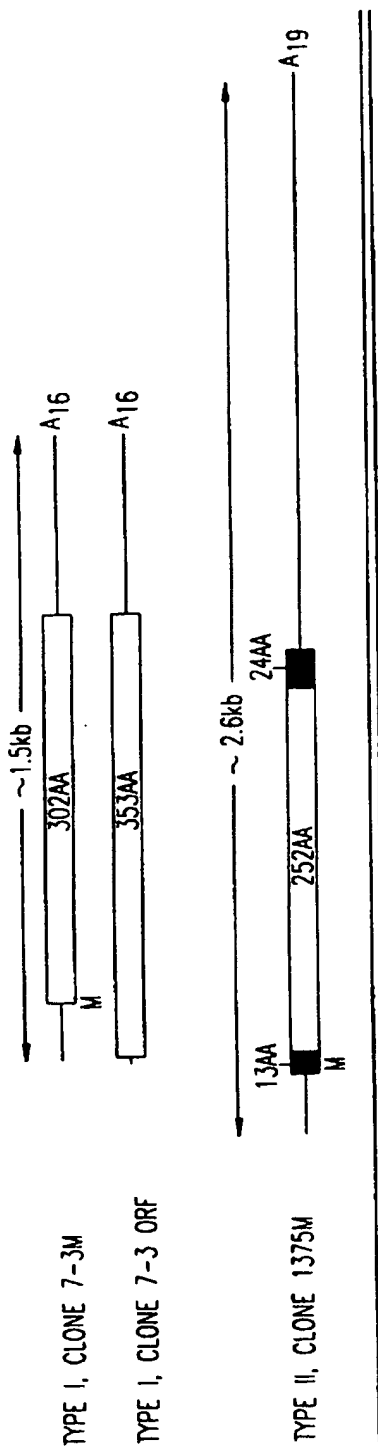
|                                 |                       |      |
|---------------------------------|-----------------------|------|
| FIG.5-SWINE TYPE II CLONE 1375m | v170                  | v180 |
|                                 | KLVILVIWAVAFCSAGPIFV  |      |
| FIG.10-HUMAN TYPE II CLONE1141m | KLVI:VIWAVAFCSAGPIFV  |      |
|                                 | KLVIFVIWAVAFCSAGPIFV  |      |
|                                 | ^170                  | ^180 |
|                                 | v190                  | v200 |
| FIG.5-SWINE TYPE II CLONE 1375m | LVGVEHDNGTDPRTNECRA   |      |
|                                 | LVGVEH:NGTDP:DTNECR:  |      |
| FIG.10-HUMAN TYPE II CLONE1141m | LVGVEHNGTDPWDTNECRP   |      |
|                                 | ^190                  | ^200 |
|                                 | v210                  | v220 |
| FIG.5-SWINE TYPE II CLONE 1375m | TEFAVRSGLLTVMVWSSVF   |      |
|                                 | TEFAVRSGLLTVMVWSS:F   |      |
| FIG.10-HUMAN TYPE II CLONE1141m | TEFAVRSGLLTVMVWSSIF   |      |
|                                 | ^210                  | ^220 |
|                                 | v230                  | v240 |
| FIG.5-SWINE TYPE II CLONE 1375m | FFLPVFCLTVLYSLIGRKLW  |      |
|                                 | FFLPVFCLTVLYSLIGRKLW  |      |
| FIG.10-HUMAN TYPE II CLONE1141m | FFLPVFCLTVLYSLIGRKLW  |      |
|                                 | ^230                  | ^240 |
|                                 | v250                  | v260 |
| FIG.5-SWINE TYPE II CLONE 1375m | RRKRGEAAVGSSLRDQNHKQ  |      |
|                                 | RR:RG:A.VG:SLRDQNHKQ  |      |
| FIG.10-HUMAN TYPE II CLONE1141m | RRRRGDAVVGASLRDQNHKQ  |      |
|                                 | ^250                  | ^260 |
|                                 | v270                  | v280 |
| FIG.5-SWINE TYPE II CLONE 1375m | TVKMLGGSQCALELSLPGPL  |      |
|                                 | TVKMLGGSQ AI I.SL:GP: |      |
| FIG.10-HUMAN TYPE II CLONE1141m | TVKMLGGSQRALRLSLAGPI  |      |
|                                 | ^270                  | ^280 |
| FIG.5-SWINE TYPE II CLONE 1375m | HSSCLFSS              |      |
|                                 | S CL::S               |      |
| FIG.10-HUMAN TYPE II CLONE1141m | I SLCLLPS             |      |

FIG.16B

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SWINE



HUMAN

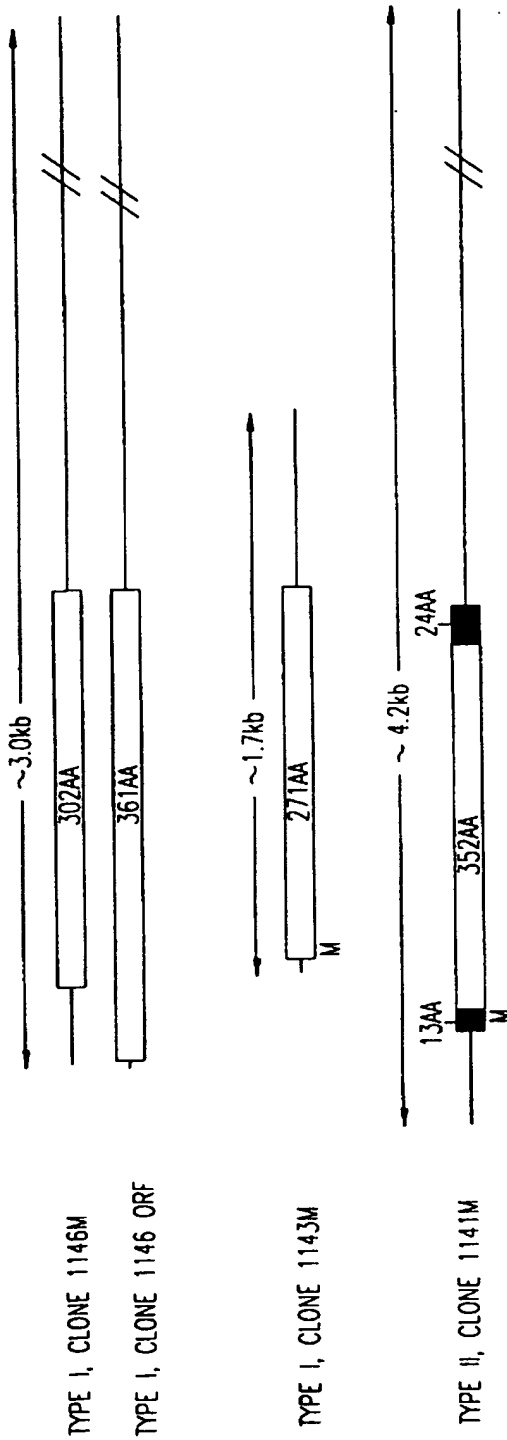


FIG.17

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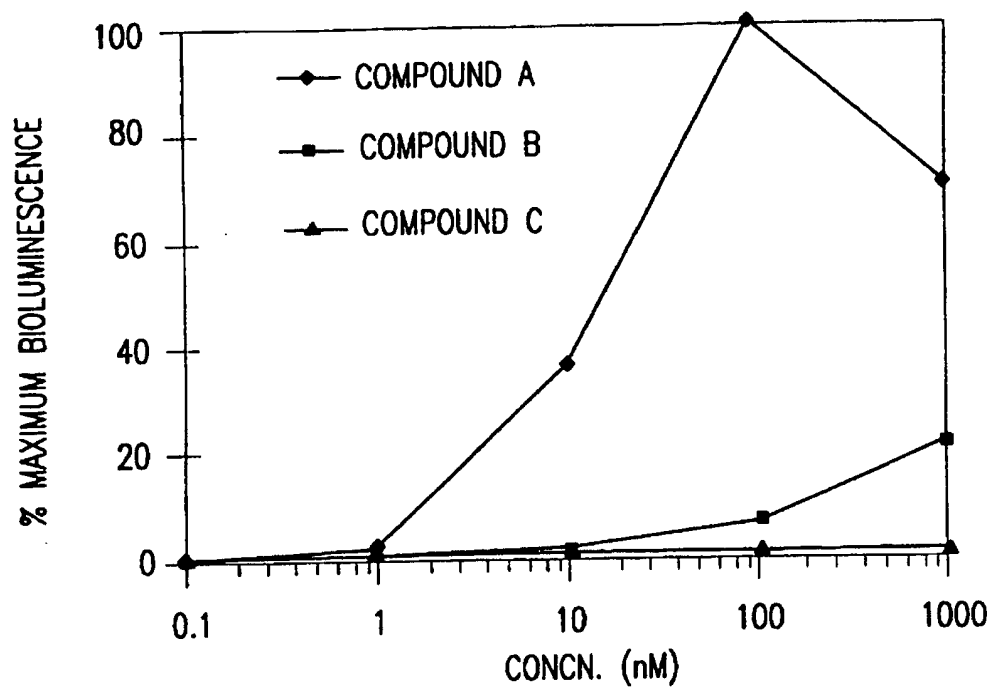


FIG.18

|  | SWINE CLONE 7-3 |                             | HUMAN CLONE 1146 |           |
|--|-----------------|-----------------------------|------------------|-----------|
|  | 24 HOURS        | 48 HOURS                    | 24 HOURS         | 48 HOURS  |
| COMPOUND A (100 $\mu$ m)<br>(1000 nM)    | 13,553<br>9,176 | 2,692                       | 1,353<br>3,091   | 2,228     |
| COMPOUND B (100nM)<br>COMPOUND C (100nM) | 717<br>100      | 425<br>58<br>3,839<br>1,806 | 113<br>96        | 108<br>67 |
| GHRP-2 (1000 nM)<br>GHRP-6 (1000 nM)     | 2,492<br>5,003  |                             | 1542<br>617      |           |

FIG.19

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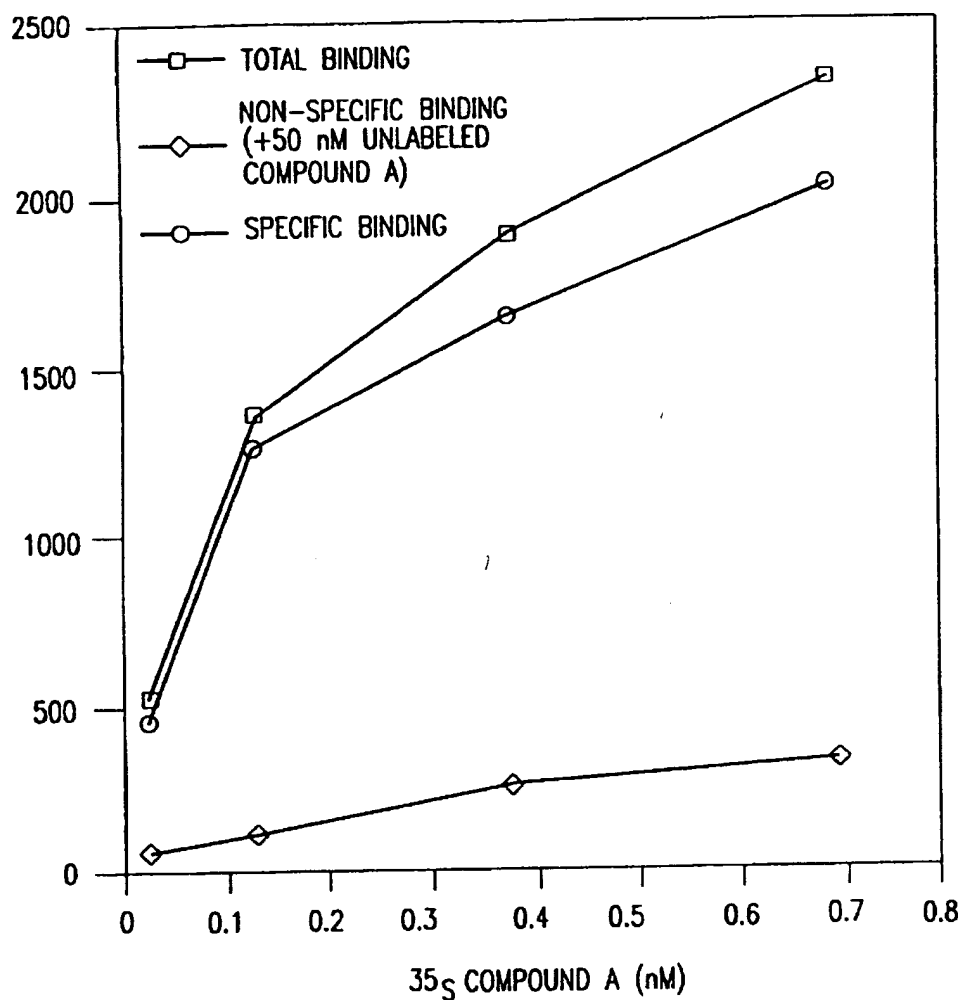


FIG.20

| LIGAND               | INHIBITION<br>(% OF CONTROL SPECIFIC BINDING) |
|----------------------|---|
| COMPOUND A @ 5nM     | 97  |
| GHRP-6 @ 10nM        | 84  |
| COMPOUND C @ 1 μM    |   |
|                      | 43  |
| GALAMIN @ 10 μM      | 44  |
| AMENOMEDIN N @ 10 μM | 19  |

FIG.21

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1 MWNATPSEEP GFNLTLADLD WDASPGNDSL GDELLQLFPA PLLAGVTATC  
51 VALFVVGIAG NLLTMLVVSF FRELRRTTNL YLSSMAFSDL LIFLCMPLDL  
101 VRI.WQYRPWN FGDLLCKLFQ FVSECTYAT VLTITALSVE RYFAICFPLR  
151 AKVVVTKGRV KLVIFVIWAV AFCSAGPIFV I VGVEHENGTD PWDNECRP  
201 TEFAVRSGLL TVMVWVSSIF FFLPVFCLTV LYSLIGRKLW RRRRGDAVVG  
251 ASLRDQNHKQ TVKMLAVVVF AFILCWLPFH VGRYLFKSKF EPGSLEIAQI  
301 SQYCNLVSEV LFYLSAAINP ILYNIMSKKY RVAVFRLLGF EPFSQRKLST  
351 LKDESSRAWT ESSINT\*

FIG.22

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|   |     |     |     |     |     |
|---|-----|-----|-----|-----|-----|
| 10  | 20  | 30  | 40  | 50  | 60  |
| ATG TGG AAC GCG ACC CCC AGC GAG GAG CCG GAG CCT AAC GTC ACG TTG GAC CTG GAT TGG |     |     |     |     | 60  |
| GAC GCT TCC CCC GGC AAC GAC TCA CTG CCT GAC GAA CTG CTG CCG CTG TTC CCC GCT CCG |     |     |     |     | 120 |
| CTG CTG GCA GGC GTC ACC GCC ACC TGC GTG GCG CTC TTC GTG GGC ATC TCA GGC AAC     |     |     |     |     | 180 |
| CTG CTC ACT ATG CTG GTG TCC CGC TTC CGC GAG CTG CGC ACC ACC AAC CTC TAC         |     |     |     |     | 240 |
| CTG TCC AGC ATG GCC TTC TCG GAT CTG CTC ATC TTC CTG TGC ATG CCG CTG GAC CTC GTC |     |     |     |     | 300 |
| 310   | 320 | 330 | 340 | 350 | 360 |
| CGC CTC TGG CAG TAC CGG CCC TGG AAC TTC GGC GAC CTG CTC TGC AAA CTC TTC CAG TTT |     |     |     |     | 360 |
| GTC AGC GAG AGC TGC ACC TAC GCC ACG GTC CTC ACC ATC ACC GCG CTG AGC GTC GAG CGC |     |     |     |     | 420 |
| TAC TTC GCC ATC TGC TTC CCT CTG CGG GCC AAG GTG GTC ACT AAG GGC CGC GTG AAG     |     |     |     |     | 480 |
| CTG GTC ATC CTT GTC ATC TGG GCC GTG GCT TTC TGC AGC GCG GGG CCC ATC TTC GTG CTG |     |     |     |     | 540 |
| GTG GGC GTG GAG CAC GAA AAC GGC ACA GAT CCC CGG GAC ACC AAC GAA TGC CGC GCC ACC |     |     |     |     | 600 |
| 610   | 620 | 630 | 640 | 650 | 660 |
| GAG TTC GCT GTG CGC TCT GGG CTG CTC ACC GTC ATG GTG TGG GTG TCC AGC GTC TTC TTC |     |     |     |     | 660 |
| TTT CTA CCG GTC TTC TGC CTC ACT GTG CTC TAC AGT CTC ATC GGG AGG AAG CTA TCG CCG |     |     |     |     | 720 |
| AGA CGC GGA GAT GCA GCG GTG GGC GCC TCG CTC CGG GAC CAG AAC CAC AAG CAG ACA GTG |     |     |     |     | 780 |
| AAG ATG CTT Ggt gag tcc tgg cac ccg ctg acc ttt ctt ccc cca ctg cct gcc ctt ccc |     |     |     |     | 840 |
| cag cgg cct cta ttt ctg ttt ctc atc atc tcc gct ccc caa gtc tct caa gtc tct gtc |     |     |     |     | 900 |

FIG.23A

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|   |      |      |      |      |      |
|---|------|------|------|------|------|
| 910   | 920  | 930  | 940  | 950  | 960  |
| ttt ctc tgc ctc tct cac ctt ggt tct cgg tct cac tgc ttt ctg ttt tct tcc tgt ctt         |      |      |      |      | 960  |
| ttc ctg tat ctt gtc cac gaa aaa gaa ccc tca tat tgg taa ttc ctt aaa acg agg aac         |      |      |      |      | 1020 |
| ctt ggt ctg gga aaa ttg gtc caa gat gga aat acc tca cgg ttt att gag ccc cta att         |      |      |      |      | 1080 |
| ggt aac ggt tta gct tct tgt ctc aca tag aat ttg tgg tta tca aag taa tat taa             |      |      |      |      | 1140 |
| ggt aag cag gca ggt aat ggg ttt aga aat cac tcc atg gta agt cta acc aca aat ttg         |      |      |      |      | 1200 |
| 1210  | 1220 | 1230 | 1240 | 1250 | 1260 |
| ggt cac tct gtt aag gac ggc tta tag atg tat ttt gtt tgt ttt caa tat tgg gat ttg         |      |      |      |      | 1260 |
| ttt tct gcc ctg cat ctt tct cag ata att aca tcc act ctg ttt agt cta tgg ttt tgc         |      |      |      |      | 1320 |
| cag gag ggg ctt cat gct ggg gtc tcc ttt ttc ttg ttt ttg tat ttg tct ccc cag taa         |      |      |      |      | 1380 |
| tat agg cca gga tag ggt gga gaa gtc atc ctt tcc tca aac tgt cct tca gga agg tct         |      |      |      |      | 1440 |
| ggg tac tga acg gtt act gca taa act ctg ctt ccc caa agg cat gtg ctt ggt gtg gta         |      |      |      |      | 1500 |
| 1510  | 1520 | 1530 | 1540 | 1550 | 1560 |
| aag tca tga aga tgg tgc tca tgt cca aga gga acc tct gat ctc act ttt caa ggg att         |      |      |      |      | 1560 |
| tca tgt ttg ctg aca ttt aat act tgt tag ttt tag ttt ttg cag ggg gat gat ttc tca ttt gca |      |      |      |      | 1620 |
| att tta tta ttc tca aat tct gca tgt cag aat gtt aga gat ttc tca ggg atg tca ggt         |      |      |      |      | 1680 |
| tct gtt tcc aga tga gtg att gcc ctg tgt cct cca ttg gac tgt aaa ctc ata tgc acc         |      |      |      |      | 1740 |
| aga cag ggt cta cat tgc tgc cgt ggt gca tag cct tcc atg tgt tgt cac tta gtc cta aag     |      |      |      |      | 1800 |

FIG.23B

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|   |      |      |      |      |      |
|---|------|------|------|------|------|
| 1810  | 1820 | 1830 | 1840 | 1850 | 1860 |
| aga agt tac taa taa cct aat ctc act aat ctc act ggc atc tca atg ccg atc cca ttg |      |      |      |      | 1860 |
| tca tct gaa aat ttg aag ggg aca tta aag tgg cac agg gac cag aac aat att ttt ctc |      |      |      |      | 1920 |
| tca ttg ctg aat ttt aaa aac aat cta aaa aat tgg aat tct tga aga aac tat ctt ata |      |      |      |      | 1980 |
| tga cta aaa tga agc ctt ggg tgg ctg cta att att gtc tgg ctt acc tgc ccc ccc     |      |      |      |      | 2040 |
| cac tac tta tat ctt tta gag atg aca cag act tgc ttt ccc tgt ggc tac taa tcc caa |      |      |      |      | 2100 |
| 2110  | 2120 | 2130 | 2140 | 2150 | 2160 |
| ttg cac att cag tcc ctt gat aga ctt act cta aaa atc taa gtt cag cgg tcc acg aaa |      |      |      |      | 2160 |
| cat aac aaa gcc tgt cct aaa aca gca aga aag aaa aga aag aaa gca aga aag aaa     |      |      |      |      | 2220 |
| gca aga aag aaa gaa aga aaa cag aag aca aac aag gtc ttt ccc cat tcc cta aca tac |      |      |      |      | 2280 |
| agg aat gga aat tat taa gtc tac gtg ata gcc aat gca tct gtt tct tca gta tgc cca |      |      |      |      | 2340 |
| caa ggg tgc tgc cgg agc cat tgc tca ggg ctg gag tat tta ctg ggc atg ctt gac ccc |      |      |      |      | 2400 |
| 2410  | 2420 | 2430 | 2440 | 2450 | 2460 |
| agc atg gag ggt gag aag tgc tcc tgg gaa ctc tga tcc act gct gtg gag agc aaa     |      |      |      |      | 2460 |
| cac ctg gcc tca ttt ata ctt gtt gtc tgt ata atg cat ata aat ggg gga taa tca tta |      |      |      |      | 2520 |
| cta aac tgt tta gct gag cct cat gtc agt caa tca aca agc aga gta att acc aca cag |      |      |      |      | 2580 |
| act ggg aag ctc agt gaa gat tgt tag cgg ttg gtc tga cag tct tgc tgt gtg cta tag |      |      |      |      | 2640 |
| tgt tag acc caa cgg agg cag tat tta toa gga ggg cag ggt tcc atg ttt ccc gtg tta |      |      |      |      | 2700 |

FIG.23C

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|   |      |      |      |      |      |
|---|------|------|------|------|------|
| 2710  | 2720 | 2730 | 2740 | 2750 | 2760 |
| aag agc aag aga tga tgt ttg tca gta ggc atg cag ctc atg gtg aaa aga aag tcc aga |      |      |      |      | 2760 |
| ctt aaa gat gtg aag tga ttt gtg ctt tgc ccc acc ctc gta gtc tct ctc tgt gtg cst |      |      |      |      | 2820 |
| tca GCT GTG GTG TTT GCT TTC ATC CTC TGC TGG CTG CCC TTC CAC GTG GGA AGA TAC     |      |      |      |      | 2880 |
| CTC TTT TCC AAG TCC TTC GAG CCT GGC TCT CTG GAG ATC GCT CAG ATC AGC CAG TAC TGC |      |      |      |      | 2940 |
| AAC CTG GTG TCC TTT GTC CTC TTC TAC CTC AGC GCT GCC ATC AAC CCC ATT CTG TAC AAC |      |      |      |      | 3000 |
| 3010  | 3020 | 3030 | 3040 | 3050 | 3060 |
| ATC ATG TCC AAG AAG TAC CGG GTG GCA GTG TTC AAA CTG CTA GGA TTT GAA TCC TTC TCC |      |      |      |      | 3060 |
| CAG AGA AAG CTT TCC ACT CTG AAG GAT GAG AGT TCC CGG GCC TGG ACA AAG TCG AGC ATC |      |      |      |      | 3120 |
| AAC ACA TGA   | 3129 |      |      |      |      |

FIG.23D

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|   |     |     |     |     |     |     |
|---|-----|-----|-----|-----|-----|-----|
| 10  | 20  | 30  | 40  | 50  | 60  |     |
| ATG TGG AAC GCG ACC CCC AGC GAG GAG CCG GAG CCT AAC GTC ACG TTG GAC CTG GAT TGG |     |     |     |     |     | 60  |
| GAC GCT TCC CCC GGC AAC GAC TCA CTG CCT GAC GAA CTG CTG CCG CTG TTC CCC GCT CCG |     |     |     |     |     | 120 |
| CTG CTG GCA GGC GTC ACC GCC ACC TGC GTG GCG CTC TTC GTG GTG GGC ATC TCA GGC AAC |     |     |     |     |     | 180 |
| CTG CTC ACT ATG CTG GTG TCC CGC TTC CGC GAG CTG CGC ACC ACC ACC AAC CTC TAC     |     |     |     |     |     | 240 |
| CTG TCC AGC ATG GGC TTC TCG GAT CTG CTC ATC TTC CTG TGC ATG CCG CTG GAC CTC GTC |     |     |     |     |     | 300 |
| 310   | 320 | 330 | 340 | 350 | 360 |     |
| CGC CTC TGG CAG TAC CCG CCC TGG AAC TTC GGC GAC CTG CTC TGC AAA CTC TTC CAG TTT |     |     |     |     |     | 360 |
| GTC ACC GAG AGC TGC ACC TAC GCC ACG GTC CTC ACC ATC ACC GCG CTG AGC GTC GAG CGC |     |     |     |     |     | 420 |
| TAC TTC GCC ATC TGC TTC CCT CTG CCG GCC AAG GTG GTG GTC ACT AAG GGC CGC GTG AAG |     |     |     |     |     | 480 |
| CTG GTC ATC CTT GTC ATC TGG GCC GTG GCT TTC TGC AGC GCG GGG CCC ATC TTC GTG CTG |     |     |     |     |     | 540 |
| GTG GGC GTG GAG CAC GAA AAC GGC ACA GAT CCC CGG GAC ACC AAC GAA TGC CGC GCC ACC |     |     |     |     |     | 600 |
| 610   | 620 | 630 | 640 | 650 | 660 |     |
| GAG TTC GCT GTG CGC TCT GGG CTG CTC ACC GTC ATG GTG TGG GTG TCC AGC GTC TTC TTC |     |     |     |     |     | 660 |
| TTT CTA CCG GTC TTC TGC CTC ACT GTG CTC TAC AGT CTC ATC GGG AGG AAG CTA TGG CGG |     |     |     |     |     | 720 |
| AGA CGC GGA GAT GCA GCG GTG GGC GCC TCG CTC CGG GAC CAG AAC CAC AAG CAG ACA GTG |     |     |     |     |     | 780 |
| AAG ATG CTT GCT GTG GTG TTT GCT TTC ATC CTC TGC TGG CTG CCC TTC CAC GTG GGA     |     |     |     |     |     | 840 |
| AGA TAC CTC TTT TCC AAG TCC TTC GAG CCT GGC TCT CTG GAG ATC GCT CAG ATC AGC CAG |     |     |     |     |     | 900 |

FIG. 24A

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |      |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|
| 910 | TAC | TGC | AAC | CTG | GTG | TCC | TTT | GTC | CTC | TTC | TAC | CTC | AGC | GCT | GCC | ATC | AAC | CCC | ATT | CTG | 960  |      |
|     | TAC | AAC | ATC | ATG | TCC | AAG | AAG | TAC | TAC | CGG | GTG | GCA | GTG | TTC | AAA | CTG | CTA | GGA | TTT | GAA | TCC  | 1020 |
|     | TTC | TCC | CAG | AGA | AAG | CTT | TCC | ACT | CTG | AAG | GAT | GAG | AGT | TCC | CGG | GCC | TGG | ACA | AAG | TCG | 1080 |      |
|     | AGC | ATC | AAC | ACA |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |      |

FIG.24B

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| 10         | 20         | 30         | 40         | 50         |     |
|------------|------------|------------|------------|------------|-----|
| MWNATPSEEP | EPNVTLDLDW | DASPGNDSLP | DELLPLFPAP | LLAGVTATCV | 50  |
| ALFVVGISGN | LLTNLVVSRF | RELRTTTNLY | LSSMAFSDLL | IFLCMPLDLV | 100 |
| RLWQYRPWNF | GDLLCKLFQF | VSECTYATV  | LTITALSVER | YFAICFPLRA | 150 |
| KVVVTKGRVK | LVILVIWAVA | FCSAGPIFVL | VGVEHENGTD | PRDTNECRAT | 200 |
| EFAVRSGLLT | VMVWVSSVFF | FLPVFCLTVL | YSLIGRKLWR | RRGDAAVGAS | 250 |
| 260        | 270        | 280        | 290        | 300        |     |
| LRDQNHKQTV | KMLAVVVFAF | ILCWLPFHVG | RYLFSKSFEP | GSLEIAQISQ | 300 |
| YCNLVSEVLF | YLSAAINPIL | YNIMSKKYRV | AVFKLLGFES | FSQRKLSTLK | 350 |
| DESSRAWTKS | SINT       | 364        |            |            |     |

FIG.25

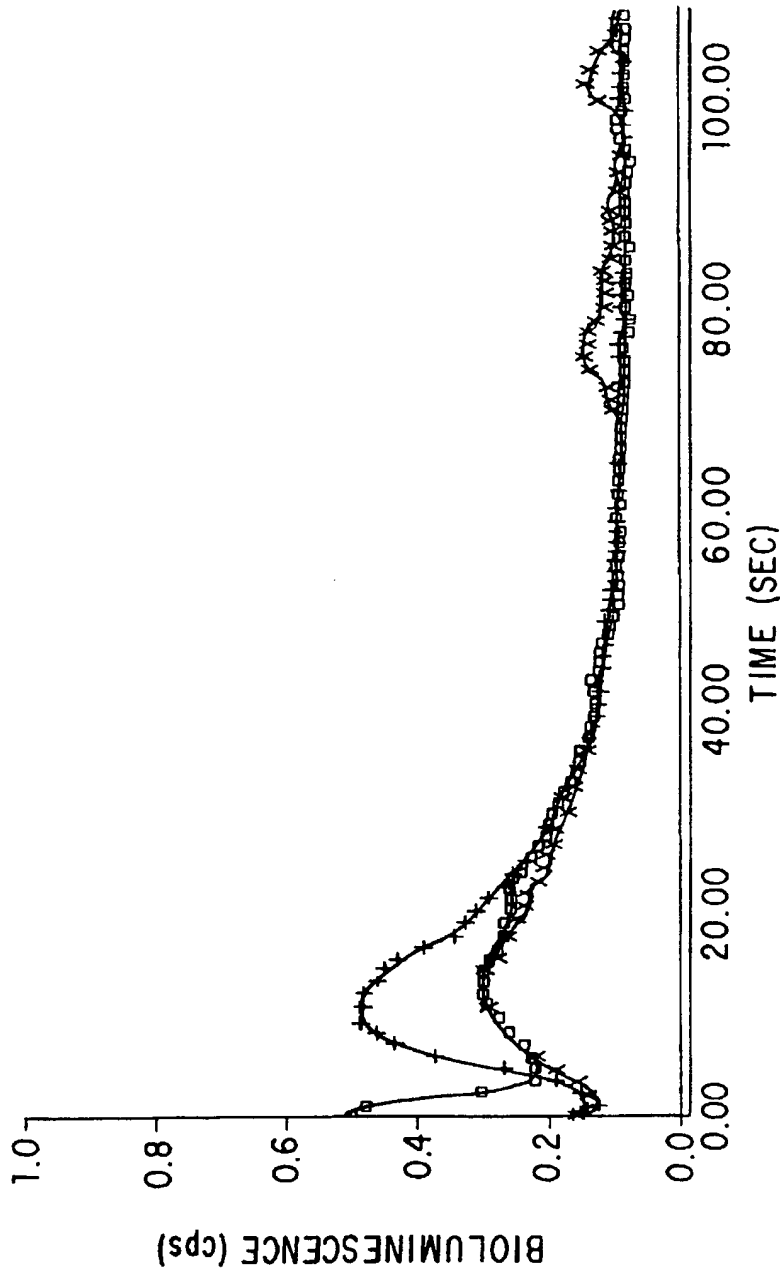


FIG. 26

## INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/US94/445

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 300; 536/23.1, 23.5; 435/69.1, 70.1, 320.1, 336, 365, 252.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, CAPLUS, WPIDS

search terms: growth hormone, receptor, secretagogue, human, rat, swine, sequence, DNA

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category*             | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.  |
|-----------------------|--|--|
| X                     | US 5,057,417 (HAMMONDS ET AL) 15 October 1991 , column 3, lines 24-39.   | 1  |
| Y, E                  | US 5,583,010 (BAUMBACH ET AL) 10 December 1996 , entire document.  | 1, 2, 4, 6, 7, 9, 14-18, 20, 22, 23, 28-30, 32, 34-36, 38, 40        |
| X, P<br>-----<br>Y, P | HOWARD ET AL. A receptor in pituitary and hypothalamus that functions in growth hormone release. Science. 16 August 1996, Vol.273, pages 974-977, see entire document. | 17-20, 22-23, 27-32, 34-38, 40<br>----- 1 -<br>10, 14-16, 21, 33, 39 |

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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| * Special categories of cited documents:  | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  |
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| *E* earlier document published on or after the international filing date  | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *G* document member of the same patent family  |
| *O* document referring to an oral disclosure, use, exhibition or other means  |  |
| *P* document published prior to the international filing date but later than the priority date claimed  |  |

Date of the actual completion of the international search

27 FEBRUARY 1997

Date of mailing of the international search report

14 APR 1997

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
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## INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/US96/19445

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|-----------|--|-----------------------|
| A         | ALOI ET AL. Neuroendocrine responses to a novel growth hormone secretagogue, L-692,429, in healthy older subjects. Journal of Clinical Endocrinology and Metabolism. October 1994, Vol.79, No.4, pages 943-949, especially last paragraph. | 1-10, 14-23, 27-40    |
| A         | BOWERS, C.Y. Editorial: On a peptidomimetic growth hormone-releasing peptide. Journal of Clinical Endocrinology and Metabolism. October 1994. Vol.79, No.4, pages 940-942.   | 1-10, 14-23, 27-40    |

# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/US96/19445

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.: 11-13 and 24-26  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
Please See Extra Sheet.
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

International application No.  
PCT/US96/19445

**A. CLASSIFICATION OF SUBJECT MATTER:**

IPC (6):

C07K 14/705, 14/72, 14/435, 14/60, 14/61, 14/47; C12N 15/12, 15/09, 15/10, 15/00, 5/10

**A. CLASSIFICATION OF SUBJECT MATTER:**

US CL :

530/350, 300; 536/23.1, 23.5; 435/69.1, 70.1, 320.1, 336, 365, 252.3

**BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE**

2. Where no meaningful search could be carried out, specifically:

Because a computer-readable copy of the sequence listing was not available, claims 11-13 and 24-26 were unsearchable to the extent that no meaningful search of the sequences per se can be carried out by this Authority. However, the subject matter of the claims has been searched to the extent possible with reference to the balance of the description.

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